INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA GOIANO – CAMPUS RIO VERDE
DIRETORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS

QUALIDADE DE SILAGEM DE CAPIM-PIATÃ ENSILADO COM DIFERENTES FARELOS DA INDÚSTRIA DE BIODIESEL

Autor: Patrícia Soares Epifânio
Orientadora: Drª Kátia Aparecida de Pinho Costa

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TITULAÇÃO: Mestre em Ciências Agrárias – Área de concentração Ciências Agrárias – Ciências Agrárias

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DEDICO

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BIBLIOGRAFIA DO AUTOR

PATRICIA SOARES EPIFÂNIO – filha de Valdeni Epifânio e Edmira Soares Epifânio nasceu em Quirinópolis, Estado de Goiás, em 18 de novembro de 1980.

No dia 09 de Setembro de 2001, iniciou no Curso de Zootecnia na Universidade Estadual de Mato Grosso do Sul – UEMS, Unidade Universitária de Aquidauana – MS, graduando em 18 de agosto de 2006. No período de 2008 a 2009 concluiu o curso de Especialização em Gestão e Produção no Setor Sucroalcooleiro e Biodiesel oferecido pela Universidade Católica de Uberlândia – MG, enfatizando o estudo dos subprodutos do setor sucroalcooleiro na alimentação animal.

No ano seguinte – 2010, ingressou no Programa de Pós-Graduação, nível Mestrado, em Ciências Agrárias do Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, atuando na área de Forragicultura, defendendo a dissertação intitulada: Qualidade de Silagem de capim-piatã ensilado com diferentes farelos da indústria de biodiesel, em fevereiro de 2012.

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EPIFÂNIO, P.S. Instituto Federal de Educação, Ciências e Tecnologia Goiano - *Campus* Rio Verde-GO, Fevereiro de 2012. **Qualidade de Silagem de capim-piatã ensilado com diferentes farelos da indústria de biodiesel** Orientadora: Prof[a]. Dr[a]. Kátia Aparecida de Pinho Costa.

A necessidade de utilização de alimentos de menor custo para ruminantes tem contribuído para aumentar a procura por novas alternativas de plantas forrageiras a serem ensiladas, sendo que, as silagens de capim se apresentam como opção promissora, sendo confeccionada a partir de um pasto já estabelecido na propriedade. Em busca de melhorar a qualidade nutricional da silagem de gramíneas, são utilizados diversos tipos de aditivos, com fontes ricas de energia e proteína, com potencial de utilização na alimentação de ruminantes. Nesse sentindo, objetivou-se avaliar as características fermentativas, bromatológicas, fracionamento da proteína e digestibilidade *in vitro* da matéria seca da silagem de capim-piatã ensilada com diferentes níveis de farelos da indústria de biodiesel. O experimento foi conduzido no Instituto Federal Goiano, Campus Rio Verde. O delineamento experimental utilizado foi o inteiramente ao acaso, com quatro repetições, em esquema fatorial 4 x 4, sendo quatro farelos oleaginosas da indústria de biodiesel (algodão, girassol, soja e canola) e quatros níveis de adição (0, 5, 10 e 15%). Os resultados demonstraram que os farelos oleaginosos da indústria de biodiesel, apresentam boa fontes de aditivos para o processo de ensilagem do capim-piatã, por trazer melhorias consideráveis nas características fermentativas, qualitativas e nutricionais da silagem de capim-piatã. Diante disso, recomenda-se a adição do nível de 15% dos farelos, por proporcionar melhor qualidade da silagem. Dentre os farelos utilizados o de soja mostrou mais eficiente para melhorar a qualidade da silagem, quando comparados aos outros aditivos.

Palavra-chave: características fermentativas, composição bromatológica, fracionamento da proteína e digestibilidade *in vitro* da matéria seca

ABSTRACT

EPIFÂNIO, P.S. Instituto Federal de Educação, Ciências e Tecnologia Goiano - *Campus* Rio Verde-GO, ("Goiano" Federal Institute of Education, Science and Technology), February de 2012. **Quality piatã grass silage ensiled with different bran oil plants** Guide: Dr[a]. Kátia Aparecida de Pinho Costa.

he need to use lower-cost food for ruminants has contributed to increase the demand for new alternative forage to be ensiled, and the grass silages are presented as a promising option because it is made from one pasture to the already established on the property. Striving to improve the nutritional quality of grass silage, has been used several types of additives, with rich sources of energy and protein, with potential use in ruminant feed. In that sense, it was aimed to evaluate the fermentation characteristics, nutritive value, protein fractionation and in vitro digestibility of dry matter grass silage ensiled with different levels Piata crumb of the biodiesel industry. The experiment was conducted at the Federal Institute of Goiás, Rio Verde Campus. The experimental design was completely randomized with four replications in a factorial scheme 4 x 4, four oilseed meals of the biodiesel industry (cotton, sunflower, soybean and canola) and four addition levels (0, 5, 10 and 15 %). The results showed that the crumbs of oilseeds biodiesel industry, have good sources of additives for silage fermentation of grass Piata, to bring considerable improvements in fermentation characteristics, and nutritional quality of grass silage-Piata. Therefore, it is recommended to add the 15% level of sharps, for providing a better quality of the silage. Among the soy bran used was more effective to improve the quality of silage, when compared with other additives.

Keywords: Fermentation characteristics, chemical composition, protein fractionation and *in vitro* digestibility of dry matter

INTRODUÇÃO GERAL

No Brasil, as gramíneas possuem grande importância, constituindo a base da alimentação dos animais dos rebanhos leiteiros e de corte (Lima & Deminices, 2008). A exploração pecuária é uma das maiores atividades econômicas brasileiras, sendo a maioria do rebanho criado em condição de pastejo, numa atividade extensiva. As áreas de pastagens compreendem aproximadamente 180 milhões de hectares, cerca de 20% do território nacional. Desse total mais de 60% das áreas pastoris são constituídas por pastagens cultivadas (IBGE, 2006).

A forte sazonalidade climática apresenta basicamente duas épocas distintas, uma de elevada e outra de reduzida precipitação pluviométrica. As gramíneas de clima tropical utilizadas nas pastagens brasileiras perdem sua qualidade e produzem muito menos nas épocas de déficit hídrico e de baixas temperaturas (Santos & Zanine, 2006). Normalmente, observa-se um excedente de forragem nas épocas das águas, que deveria ser conservado, para posterior fornecimento nas épocas mais secas do ano (Santos et al., 2006).

Dentre as gramíneas tropicais, destacam-se as do gênero *Brachiaria*. Aproximadamente 85% das áreas de pastagem cultivadas no país são ocupadas por espécies desse gênero (Macedo, 2005).

No Brasil, foram encontradas 16 espécies deste gênero, das quais cinco são nativas, três foram provavelmente introduzidas há várias décadas, sendo, portanto consideradas como naturalizadas, e sete foram introduzidas recentemente, sendo cultivadas como forrageiras (Seiffert, 1980). Com isso, visando a sustentabilidade do sistema de produção com alta produtividade dos componentes planta e animal, novos cultivares vêm sendo lançados no mercado para atender a necessidade do sistema e do produtor. Sendo assim, a Embrapa Gado de Corte lançou a *Brachiaria brizantha* cv. Piatã, como mais uma opção na diversificação de forragem (Embrapa, 2008a).

A *Brachiaria brizantha* cv. Piatã é a primeira forrageira protegida lançada pela Embrapa, foi desenvolvida a partir da coleção de forrageiras da Empresa e passou por avaliações durante 16 anos (Embrapa, 2008b). Além disto, é uma planta apropriada para solos de média fertilidade, tolera solos mal drenados, produz forragem de boa qualidade por causa da boa porcentagem de folhas e colmos finos o que resulta em um melhor aproveitamento pelo animal. Sendo resistente ao ataque de cigarrinhas-das-pastagens e se destaca pelo elevado valor nutritivo e alta taxa de rebrotação (Embrapa, 2007). Diante dessas características, o capim-piatã também está sendo utilizado para a produção de silagem (Costa et al., 2011).

Segundo Cardoso e Silva (1995) conceituam a silagem como sendo a forragem verde, suculenta, conservada por meio de um processo de fermentação anaeróbica, isto é, na ausência de oxigênio sendo que, as silagens são guardadas em silos, e a ensilagem se caracteriza como o processo de cortar a forragem, colocar no silo, compactar e proteger com a vedação do silo para que haja a fermentação. Diante disto, Balsalobre et. al. (2001) relataram que silagem de gramíneas tropicais é uma boa alternativa para aumentar o estoque de forragem para seca, particularmente para categorias menos exigentes, ou para regiões que disponham de concentrados baratos. A possibilidade de mais de um corte por ano, e posterior aproveitamento do rebrote para pastejo podem compensar as dificuldades encontradas na confecção das silagens de capins (Jayme et al., 2009).

Outro fator importante a ser considerado quando se trata de silagem de capim é o menor custo quando comparada com silagem de milho e sorgo ou outras fontes de suplementação volumosa. Mesmo não sendo uma prática recente, o uso de silagem de gramíneas tropicais, somente vem ganhando espaço, nos últimos anos, desde que se utilizem as técnicas de confecção adequadas, no sentido de se reduzirem as perdas e melhorar a qualidade das silagens (Santos et al., 2006).

Um dos pontos importantes para a ensilagem é o corte das plantas forrageiras. Esse corte deve ser realizado no estádio vegetativo em que a planta se encontra no seu “ponto de equilíbrio” entre produção de massa seca e qualidade nutricional (Jayme et al., 2009), com partículas de 3 a 5 cm, facilitando a compactação e a fermentação (Moraes, 2002). Estes cuidados são importantes já que, as gramíneas tropicais apresentam alto teor de umidade e baixo teor de carboidratos solúveis no momento do corte, inibindo o processo fermentativo e dificultando a confecção de silagens de boa qualidade (Galan & Nússio, 2000).

Paziani et al. (2006), relataram que o valor nutritivo da silagem dependerá, além do processo de conservação, da composição da forragem ensilada, uma vez que a ensilagem não melhora a qualidade nutricional da forragem original, sendo importante a utilização de bom aditivos.

Segundo Pizarro (1978), aditivos são substâncias, misturas ou combinações destas que quando adicionadas às forragens no momento da ensilagem podem promover melhorias na sua fermentação, aceitabilidade e valor nutritivo. Entretanto, é fundamental lembrar que a utilização de aditivos não elimina os cuidados normais para obtenção de boas silagens (época de corte, compactação da forragem, vedação do silo etc.). Deve-se, também, considerar alguns fatores em relação aos aditivos: custo e facilidade de aplicação, eficiência na fermentação e melhoria do valor nutritivo. Assim, o sucesso na utilização de aditivo depende da escolha de um material que atenda o grande porte dessas condições.

De acordo com Bergamaschine et al. (2006), os aditivos utilizados nas silagens de capim deve apresentar alto teor de matéria seca, alta capacidade de retenção de água, boa palatabilidade, além de fornecer carboidratos para a fermentação. Estes tipos de aditivos limitam a ação de bactérias do gênero *Clostridium* e elevam o teor de açúcares na massa ensilada, facilitando o estabelecimento das bactérias que produzem ácido lático (Bernardes et al., 2005).

Segundo McDonald et al. (1991), os aditivos são classificados em estimulante da fermentação no silo, inibidores da deterioração aeróbia, nutritivos, ou seja, aqueles que acrescentam nutrientes ao material ensilado e absorventes de umidade. Vilela (1998) faz ressalva de mais um grupo, aquele que associa mais de um efeito, como os que estimulam a fermentação e que são nutritivos.

Em busca de novas alternativas como fonte de aditivos, o aproveitamento do alimento da agroindústria na alimentação animal vem sendo muito difundida. A utilização dos subprodutos da agroindústria é avaliada por diversos autores (Bernardes et al., 2005; Santos, et al., 2010, Oliveira et al., 2011), que se centraram em determinar quais percentuais aditivos podem ser adicionados na silagem, com o objetivo de promover melhoria nas condições de fermentação da silagem, principalmente pelo incremento do teor de matéria seca.

Os produtos de agroindústrias, principalmente os de processamento de grãos de cereais oleaginosos para extração do óleo, produzem um subproduto (farelos) que pode ser utilizado como aditivos nas silagens de capins por conter alto valor nutricional (Muraro et al., 2008). Os farelos são obtidos após a extração por solventes, possibilitando a obtenção de um material com baixo teor de óleo (menos que 1,5%), assim resultando em um maior teor de proteína bruta (Evangelista et al., 2004).

Dentre os vários farelos da indústria de biodiesel existentes e que podem ser utilizados na ensilagem de gramíneas estão os farelos de algodão, girassol, soja e canola, sendo que, ambos podem variam em quantidade e qualidade conforme a região. No Estado da Goiás, encontra-se em quantidades significativas, o farelo de soja, que pode ser utilizado como aditivos para ensilagem de gramíneas tropicais com a finalidade de melhorar o processo fermentativo, além de reduzir as perdas por gases e efluente.

O termo qualidade de silagem se refere a eficiência do processo fermentativo para promover a conservação do valor nutritivo da forragem ensilada. Entre os principais parâmetros utilizados para avaliar a qualidade do processo fermentativo, estão as características químicas apresentadas pelas silagens, como teor de matéria seca, valor de pH e o conteúdo de amônia, expresso como porcentagem do nitrogênio amoniacal em relação ao nitrogênio total ($N-NH_3/NT$) entre outros parâmetros.

Essas características juntas fornecem uma indicação da forma como se processou a fermentação. De acordo com Haigh (1999), o teor de MS desempenha papel fundamental na confecção da silagem ao aumentar a concentração de nutrientes, e facilitar os processos fermentativos diminuindo a capacidade de ação dos *clostrídios*, além disto, o pH elevado também contribui para a produção deste tipo de bactérias indesejáveis ao processo final de fermentação. De acordo com Van Soest (1994), baixos teores de $N-NH_3/NT$ na silagem, inferior a 10% do nitrogênio total, indica que o processo de fermentação não resultou em quebra excessiva da proteína em amônia, sendo que, acima de 15% do nitrogênio total ocorre a degradação dos compostos proteicos, resultando em baixo consumo.

O valor nutritivo também pode ser predito através da sua composição bromatológica, principalmente pelos teores de proteína bruta (PB), fibra detergente neutro (FDN), fibra detergente acido (FDA), celulose, hemicelulose e lignina (Silva & Queiroz, (2002). Além disso, vem sendo estudados novos sistemas e metodologias de avaliação de alimentos para ruminantes, com intuito de maximizar o uso dos nutrientes pelos animais (Balsalobre et al., 2003).

O *Cornell Net Carbohydrate and Protein System* é um sistema que considera a dinâmica da fermentação ruminal e a perda potencial de nitrogênio, como amônia, na avaliação dos alimentos (Sniffen et al., 1992) e tem por objetivo adequar a digestão ruminal dos carboidratos e das proteínas, visando maximizar a produção microbiana, a redução das perdas do nitrogênio pelo animal e estimar o escape ruminal de nutrientes (Balsalobre et al., 2003).

Nesse sistema a proteína é dividida em frações A, B1, B2, B3 e C. A fração "A" representa a fração da proteína que é instantaneamente solubilizada no rúmen, sendo constituída de nitrogênio não prot\eico (NNP). A fração "B" representa a proteína verdadeira potencialmente degradável, sendo dividida em três subfrações, baseada na velocidade de degradação ruminal. A fração "B1" é a fração da proteína bruta do alimento que é rapidamente degradável no rúmen, "B2" com taxa de degradação intermediária, "B3" é a proteína associada à parede celular e de degradação lenta e, por fim, a fração "C", que é composta de proteínas insolúveis em detergente ácido, ou seja, que não é digerível no rúmen e intestino (Sniffen et al., 1992).

Assim, a caracterização das frações proteicas dos alimentos representa um importante instrumento para adequação de dietas que visem a maximização do desenvolvimento microbiano ruminal e, por consequência, a melhor predição do desempenho animal (Balsalobre et al., 2003). Vale ressaltar que as frações desejáveis são: os maiores teores da fração A e B1 e menor teor para a fração C, assim proporcionando melhor digestibilidade.

Ainda é de grande valia o conhecimento da utilização dos nutrientes pelo animal, que é obtido por meio de estudos de digestibilidade e de degradabilidade ruminal e, é de fundamental importância para expressar o valor nutricional de um alimento. Dentre as técnicas empregadas para avaliar a degradação ruminal, o método *in vitro* é capaz de representar o processo de digestão que ocorre no rúmen, abomaso ou intestino para estimar quantitativamente a taxa e o grau de digestão, de forma semelhante ao que acontece *in vivo* (Berchielli et al., 2006).

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OBJETIVOS GERAIS

O objetivo do trabalho foi avaliar a qualidade da silagem de capim-piatã ensilado com diferentes farelos da indústria de biodiesel, através das características fermentativas, bromatológicas, fracionamento da proteína e digestibilidade *in vitro* da matéria seca.

CAPITULO 1

Piatã grass ensiled with different levels of meals from biodiesel industry: fermentation and bromatological characteristics

ABSTRACT: Tropical grasses silage is indicated to increase the forage stock during the drought. But the high humidity and low content of soluble carbohydrates at the time of cutting of tropical grasses inhibit an adequate fermentative process, hindering the achievement of good quality silages. Alternatives to increase the content of dry matter and inclusion of soluble carbohydrates into the material to be ensiled have been extensively studied, by using additives that provide good quality silages. Among these additives, stand out the meals of oleaginous plants, rich sources of energy and protein. In this way, this study evaluated the fermentative and bromatological characteristics of piatã grass ensiled with different levels of meals from biodiesel industry. The experiment was performed at the Federal Institute of Goiás State, Campus Rio Verde. The experimental design was completely randomized with four replications, in 4 x 4 factorial scheme, being four meals (cotton, sunflower, soybean, and canola) and four levels of inclusion (0, 5, 10 and 15%). The results have demonstrated that the meals from biodiesel industry have a good source of additives for ensilaging process, by improving fermentative, qualitative and nutritional characteristics of the piatã grass. It is recommended the addition of 15% of the meals, once this level provided the best quality silage. The soybean meal proved to be the most effective to improve the silage quality.

KEY WORDS: additive, fibrous materials, ammonia nitrogen, crude protein

RESUMO: A silagem de gramíneas tropicais é indicada como alternativa para aumentar o estoque de forragem para seca. No entanto, a alta umidade e baixo teor de carboidratos solúveis no momento do corte das gramíneas tropicais, são fatores que inibem um adequado processo fermentativo, dificultando a confecção de silagens de boa qualidade. Alternativas para aumentar o teor de matéria seca e o aporte de carboidratos solúveis no material a ser ensilado têm sido amplamente estudadas, através do uso de aditivos, que proporcionam uma silagem de melhor qualidade. Dentre esses aditivos se destacam os farelos de plantas oleaginosos que apresenta fontes ricas de energia e proteína. Nesse sentindo, objetivou-se avaliar as características fermentativas e bromatológicas da silagem de capim-piatã ensilada com diferentes níveis de farelos da indústria do biodiesel. O experimento foi conduzido no Instituto Federal Goiano, Campus Rio Verde. O delineamento experimental utilizado foi o inteiramente ao acaso, com quatro repetições, em esquema fatorial 4 x 4, sendo quatro farelos (algodão, girassol, soja e canola) e quatros níveis de adição (0, 5, 10 e 15%). Os resultados demonstraram que os farelos da indústria do biodiesel apresentam boa fonte de aditivos para a ensilagem, por trazer melhorias nas características fermentativas, qualitativas e nutricionais da silagem de capim-piatã. Recomenda-se a adição do nível de 15% dos farelos, por proporcionar melhor qualidade da silagem. O farelo de soja mostrou mais eficiente para melhorar a qualidade da silagem, quando comparados com os outros aditivos.

Palavras-chave: aditivo, fração fibrosa, nitrogênio amoniacal, proteína bruta.

Introduction

The seasonality in the forage production in the cerrado has been limiting for ruminant production, due to the low and unevenly distributed rainfall. This seasonality reduces the livestock yield by significantly reducing the supply of forage during the dry period. Thus the low quality and quantity of forage in this period require the use of practices to preserve the food produced in the rainy period, like the ensilaging process (Regô et al., 2010).

New cultivars have been continuously created seeking a high productivity of the plant and animal, in order to meet the requirements of the different production systems, with varied levels of technology. Thus, the Embrapa Beef Cattle launched the *Brachiaria brizantha* cv. Piatã, as an option for forage diversification (Embrapa, 2008). This grass has an excellent performance on soils of medium fertility, high regrowth rate and high production of forage with high nutritional value (Embrapa, 2008). Besides being used for grazing, it has nutritional characteristics suitable for making silage (Costa et al., 2011).

Therefore, the high humidity at the ideal time for cutting, the low content of soluble carbohydrates, and the high buffering capacity of this grass, inhibit an adequate fermentative process, hindering the production of a good quality silage (McDonald, 1991). These factors negatively influence the fermentative process, hindering the rapid decrease in pH, allowing an undesirable secondary fermentation (Evangelista et al., 2004).

Given these difficulties, the grass silage requires additives to provide a good quality silage. According to Santos et al. (2010) a good additive to the silage of tropical grasses should present a high content of dry matter, disfavoring the growth of yeasts and contributing to lower losses of efluents, and with high nutritional level, palatability, and high content of soluble carbohydrates, as well as easy handling, good availability in the market and low cost. These additives limit the action of bacteria of the genus *Clostridium*, increase the sugar content in the silage, easing the establishment of bacteria producers of lactic acid

Among several alternatives of natural additives, the oleaginous meals from biodiesel production have emerged recently, such as meals of cotton, sunflower, soybean and canola. These meals are sources rich in energy and protein, representing an alternative to improve the silage quality, by correcting the low contents of dry matter and contributing to a better fermentation of the silage. But little is known on the effect of these meals on the fermentative and chemical parameters of the tropical grass silage. In this way, this study assessed the fermentative and bromatological characteristics of piatã grass ensiled with different levels of meals from biodiesel industry.

Material and Methods

The experiment was conducted in the Federal Institute of Goiás State, Campus Rio Verde, 748 m altitude, 17° 48' south latitude and 50º 55' west longitude, from September 2010 to July 2011. The area of pasture used to produce the silage had 180 m^2.

The soil was classified as distroferric Red Latosol (Oxisol), with 530 g/kg clay; 250 g/kg silt and 220 g/kg sand. The chemical characteristics of the soil at the layer 0-20 cm, before planting were: pH in water: 5.6; Ca: 4.04 $cmol_c/dm^3$; Mg: 2.0 $cmol_c/dm^3$; Al: 0.0 $cmol_c/dm^3$; Al+H: 6.6 $cmol_c/dm^3$; K: 65 mg/dm^3; CTC: 7.05 $cmol_c/dm^3$; P: 8.07 mg/dm^3; Cu: 3.7 mg/dm^3; Zn: 1.8 mg/dm^3; V: 48.4%; M.O: 35.6 g/kg.

The area was prepared with harrowing followed by leveling. By the planting of forage it was applied 80 kg/ha P_2O_5, using the super triple phosphate. Then, the piatã grass was broadcast seeded with 9 kg viable pure seeds per hectare. The standardization cut was held at 40 days after planting, and the topdressing included 80 kg/ha nitrogen and 40 kg/ha potassium, as urea and potassium chloride, respectively.

The experimental design was the completely randomized with four replications in a 4 x 4 factorial scheme, being four meals from biodiesel industry (cotton, sunflower, soybean, canola) and four levels of inclusion (0, 5, 10 and 15%). The meals were obtained from mechanical extraction of oil, where the amount applied was based on the natural material of piatã grass.

For the ensilaging process, the piatã grass was harvested at 45 days after maintenance fertilization, at 20 cm from ground level, using backpack crush cutter. Afterwards, the forage was minced into 10-30mm-particles, with a stationary shredder, and ground. Then, the material was homogenized with the meals, according to the different levels determined, and stored in PVC experimental silo, with 10cm diameter and 40cm length.

The silage was compacted with iron pendulum and the silos were sealed with PVC caps and adhesive tape to preclude the entry of air. Immediately after, they were stored at room temperature and protected from rain and sunlight.

After 60 days of fermentation, the silos were opened, discarding the top and bottom portion of each. The central portion was homogenized and placed into plastic trays. Part of the fresh silage after opening the silo was set apart to analyze the fermentation parameters, such as pH, titratable acidity, and ammonia nitrogen in relation to total nitrogen ($N\text{-}NH_3/NT$). The determination of pH and titratable acidity of the silage was made using potentiometer Beckman Expandomatic SS-2 after opening up the silos.

After this, the silage was split into two portions. The first was placed into plastic bags and frozen. For the determination of ammonia nitrogen [N-NH_3 (% total N)] the samples were thawed to extract the juice, with press (AOAC, 1980). The other part of about 1 kg was weighed and taken to a forced air oven at 55ºC for 96 hours to determine the pre-drying matter. Later, the samples were ground in a Willey type mill, with 1mm-sieve, to be analyzed.

As for the chemical and bromatological composition of the silages, it was determined the contents of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose, hemicellulose, ether extract (EE), and ash, by the method described by Silva and Queiroz (2002). The total carbohydrates (TCH) was obtained by the equation of Sniffen et al. (1992): TCH = 100 – (% CP + % EE + % ash).

Before the ensiling process, the piatã grass and the meals were submitted to bromatological analysis, as shown in Table 1.

Table 1. Bromatological composition of the piatã grass and meals of cotton, sunflower, soybean, and canola, used to produce the silage.

Bromatological composition	**Piatã grass**	**Cotton**	**Sunflower**	**Soybean**	**Canola**
DM (g/kg)	184.0	901.5	911.3	887.2	890.6
CP (g/kg)	125.5	417.0	286.1	420.4	379.5
NDF (g/kg)	683.2	230.3	454.0	180.7	321.0
ADF (g/kg)	395.0	149.3	235.2	102.4	223.5
Lignin (g/kg)	49.1	43.9	41.8	37.8	41.6
Cellulose (g/kg)	346.0	105.3	193.5	64.6	181.2
Hemicellulose (g/kg)	288.3	81.5	219.0	78.3	98.0
EE (g/kg)	23.5	26.3	21.55	34.3	25.6
Ash (g/kg)	113.1	95.4	102.0	87.5	100.3
Total CH (g/kg	738.0	441.5	591.8	459.3	496.2
TDN (g/kg)	594.2	694.1	570.0	740.5	640.3

Data were subjected to analysis of variance and regression analysis, and the mean values were compared by the Tukey's test at 5%, using the statistical software SISVAR 4.6 (Ferreira, 2000).

Results and Discussion

The contents of DM, pH, titratable acidity, $N\text{-}NH_3/TN$, EE, MM, TCH, CP, NDF, ADF, lignin, cellulose, and hemicellulose were influenced ($P<0.05$) by the additives, levels, and interaction between these factors (Tables 2, 3 and 4).

The pH of a food is one of the major factors determining the proliferation and survival of microorganisms, besides being used as a quality parameter of the silage (Amaral et al., 2007). There was a quadratic decrease of pH for all aditives, by adding the meals in the silage, with minimum values of 3.8; 3.6; 3.6 and 4.2 for the levels of 14.1; 13.9; 14.5 and 14.7%, for the meals of cotton, sunflower, soybean, and canola, respectively. For all used additives these levels were more effective in decreasing the silage pH. This result is related to the greater contents of DM at this level, since they have a high content of DM in relation to piatã grass (Table 1). According to Jobim et al. (2007), silage of materials with high humidity has high pH values (above 4.2). Thus, these results suggested a suitable fermentation of the silage mixtures, a desirable feature for the ensiling process. This allows inferring that by adding the meals from biodiesel industry, there is a suitable amount of soluble carbohydrates to the action of lactic bacteria, which leads to lower pH.

Table 2. Fermentative parameters of piatã grass ensiled with different levels of additives.

Additives	**Additive Levels**				**Equation**	R^2
	0	**5**	**10**	**15**		
	Values of pH					
Cotton	4.9 a	4.2 b	3.9 b	3.8 b	$Y = 4.8900 - 0.1620x + 0.0060x^{2*}$	0.99
Sunflower	4.9 a	4.1 b	4.0 b	3.6 c	$Y = 4.8025 - 0.1295x + 0.0035x^{2*}$	0.94
Soybean	4.8 a	3.8 b	3.7 b	3.6 c	$Y = 4.7550 - 0.2090x + 0.0090x^{2*}$	0.95
Canola	5.1 a	4.7 a	4.4 a>	4.2 a	$Y = 5.1000 - 0.0900x + 0.0020x^{2*}$	0.99
CV (%)	.... 6.06					

Titratable acidity

Cotton	10.4 a	11.0 b	10.8 b	11.1 b	ns	
Sunflower	11.4 a	11.8 b	11.4 b	11.5 b	ns	
Soybean	12.3 a	20.1 a	22.5 a	22.6 a	$Y = 11.7900 + 1.9680x - 0.0840x^{2*}$	0.99
Canola	9.5 a	9.7 c	9.2 c	8.9 b	ns	
CV (%)	.... 14.03					
	Content of $N\text{-}NH_3$ (g/kg total N)					
Cotton	58.0 a	47.3 a	45.1 a	36.5 a	$Y = 57.160 – 1.3680x$	0.93
Sunflower	56.2 a	46.0 a	40.3 b	33.2 b	$Y = 55.140 – 1.5020x$	0.98
Soybean	57.1 a	41.3 a	37.0 c	29.5 c	$Y = 54.750 – 1.770x$	0.94
Canola	57.0 a	47.2 a	43.5 a	41.0 a	$Y = 57.620 – 2.356x + 0.0880x^{2*}$	0.98
CV (%)	.... 8.33					

Means followed by different lowercases in the column (additive) are significantly different by Tukey's test (P<0.05). *significant at 0.05 level, ns = non-significant at 0.05 level.

Evaluating the additives within each level, at the level zero the pH values were similar between the studied additives. At the levels of 5, 10 and 15% the canola meal achieved the highest values of pH, proving to be more effective for lowering the silage pH, when compared to the other additives, with a reduction of 0.9 percentage units in relation to level zero, while the average of the other meals was 1.2 percentage units (Table 2).

Tomich et al. (2004) reported that pH between 3.8 and 4.2 are considered suitable to well preserved silages, once these pH values sufficiently restrict the proteolytic enzymes of the plant, and of enterobacteria and *Clostridium*, to preserve the material. Also, at 15% level, the soybean and sunflower meals presented the lowest pH values. Paziani et al. (2006) found a value of 4.7 when added millet meal in tanzania-grass silage.

Only the soybean meal influenced the titratable acidity (Table 2), as added the additive levels to the silage, it increased quadratically, with a maximum at the level of 14.8%, indicating an increase of 11.0 percentage units in relation to level zero. However, the meals of cotton and sunflower were not mutually different, and the canola meal promoted the lowest titratable acidity.

At the levels of 5 and 10%, the soybean meal presented higher values of titratable acidity, and the canola meal, the lowest ones, differing from cotton and sunflower meals. At the 15% level, only the soybean meal was different from the others.

Silva and Queiroz (2002) observed that the types of additives may interfere with the relationship between pH and lactic acid, and the titratable acidity indicates the general aspect of the fermentation quality of silages, influencing the taste, odor, color, and stability, by being directly related to the acids that determine the pH, primarily lactic acid (Nussio et al., 2001).

The content of N-NH_3/TN also indicates the silage quality and assists in characterizing the fermentation during the process. The lower this ratio, the lower the proteolysis of ensiled material and the best quality silage (McDonald et al., 1991). A linear decrease was found in N-NH_3/TN for cotton, sunflower and soybean meals as increased the additive levels in the silage. Nevertheless, the canola meal caused a quadratic reduction, with a minimum observed at the level of 13.9%. The inclusion of 15% promoted a reduction of 22; 23; 27 and 16 percentage units in relation to the non-addition of this meal, indicating a decrease in the degradation of crude protein. This is due to the higher content of DM and high pH at the level 15%, which may reduce the activity of bacteria of the genus *Clostridium*, which promote proteolysis and release of N-NH_3/TN during the ensiling process (Teixeira et al., 2008). However, the greater content of N-NH_3/TN in the silage without additives was due to the lower content of readily fermentable carbohydrates, the lower DM content and higher buffering capacity, typical of perennial grasses (Leonel et al., 2009).

At levels zero and 5% the contents of N-NH_3/TN were similar between the meals examined. At the levels 10 and 15%, the soybean meal presented the lowest content. But all the silages had values within the recommended range for a good quality silage. Even the treatments without additives, the contents of N-NH_3/TN were acceptable, with values ranging from 562.0 to 588.0 g/kg. These have remained below 100 g/kg indicating the good quality of the silage for this parameter according to Tomich et al. (2004). This indicates that the fermentation did not resulted in excessive breakdown of protein into ammonia, and the amino acids are most of non-protein nitrogen (Van Soest, 1994).

Oliveira et al. (2010) found N-NH_3/TN between 24.0 and 58.0 g/kg relative to 1^{st} and 56^{th} days of fermentation, respectively, when added 10% of palm cake in the pupunha byproduct silage. By evaluating the fermentative characteristics of elephant-grass silage added with cocoa meal, Carvalho et al. (2008) observed a quadratic effect of the inclusion levels of the meal on the contents of N-NH_3/TN, with the minimum value (28.3 g/kg) in the silage with 4.5% of cocoa meal.

The content of DM increased linearly with the levels of all meals in the silage (Table 3), showing the efficiency of these additives to absorb water inside the silo, improving thus the fermentation and the silage quality. Brito et al. (2000) reported that the content of DM of the silage is important to determine the type of predominant fermentation in the ensiling process.

At the level of 5%, only the soybean differed from the other treatments with higher content of DM. At the level of 15%, the content of DM was similar between the meals of cotton, sunflower and soybean, only differing from canola meal, with the highest value, increasing 9.8 percentage units in relation to the control (Table 3).

The addition of 8, 16 and 24% of palm cake from biodiesel industry to massai grass silage promoted a great absorption of humidity, increasing linearly the content of DM from 223.0 to 381.0 g/kg at the levels zero to 24%, respectively (Oliveira et al., 2011). By adding 15% of millet meal, Costa et al. (2011) registered increases in the DM content from 205.0 to 284.0 g/kg; from 219.0 to 315.0 g/kg, and from 197.0 to 310.0 g/kg DM for the silages of marandu, piatã and xaraés grasses, respectively. In the same way, Gonçalves et al. (2007) evaluated the elephant-grass silage added with cashew apple and achieved a remarkable increase in DM content, varying from 208.0 g/kg at level zero, to 338.0 g/kg by adding 20% of the additive.

Table 3. Chemical and bromatological composition of piatã grass ensiled with different levels of cotton, sunflower, soybean and canola meals.

Additives	**Additive Levels**				**Equation**	**R^2**
	0	**5**	**10**	**15**		
	Content of DM (g/kg)					
Cotton	171.2 a	184.5 b	200.3 a	217.0 b	$Y = 169.9000 + 3.0800x^*$	0.99
Sunflower	169.0 a	180.3 b	196.0 a	214.2 b	$Y = 167.100 + 3.0200x^*$	0.98
Soybean	168.5 a	208.4 a	217.5 a	234.0 b	$Y = 175.700 + 4.1400x^*$	0.91
Canola	171.3 a	187.0 ab	209.4 a	269.3 a	$Y = 161.600 + 6.3200x^*$	0.90
CV (%)	.... 6.89					
	Content of CP (g/kg)					
Cotton	79.0 a	146.2 a	184.5 a	208.7 a	$Y = 90.500 + 8.5000x^*$	0.95
Sunflower	77.3 a	121.4 b	142.7 b	164.0 b	$Y = 83.700 + 5.6400x^*$	0.96
Soybean	78.5 a	155.0 a	211.3 a	232.5 a	$Y = 91.300 + 10.3600x^*$	0.94

Canola	78.1 a	106.3 b	127.5 c	170.0 b	Y = 76.050 + 5.9100x*	0.97
CV (%)	.... 16.81					
Content of EE (g/kg)						
Cotton	25.0 a	28.2 b	38.5 a	41.3 b	Y = 24.300 + 1.1600x*	0.94
Sunflower	27.2 a	42.5 a	44.0 a	52.3 a	Y = 29.735 + 1.5470x*	0.90
Soybean	28.3 a	33.5 b	40.2 a	42.6 b	Y = 28.400 + 0.9800x*	0.96
Canola	24.5 a	41.0 a	43.4 a	53.2 a	Y = 26.800 + 1.8100x*	0.91
CV (%)	.... 16.81					
Content of MM (g/kg)						
Cotton	105.2 a	100.5 a	93.4 a	92.5 a	Y = 104.470 – 0.8760x*	0.91
Sunflower	112.0 a	101.3 a	96.0 a	85.3 b	Y = 112.170 – 1.7360x2*	0.97
Soybean	105.5 a	87.5 a	75.5 b	69.4 b	Y = 102.310 – 2.4080x*	0.95
Canola	110.0 a	82.3 a	80.2 b	77.0 b	Y = 108.6650 - 5.6970 + 0.2450x2*	0.94
CV (%)	.... 17.94					
Content of TCH (g/kg)						
Cotton	794.0 a	743.2 b	709.3 b	677.1 b	Y = 788.810 – 7.6880x2*	0.98
Sunflower	782.5 a	731.4 b	718.0 b	697.2 b	Y = 772.590 – 5.3420x2*	0.91
Soybean	786.2 a	730.5 b	681.3 b	665.0 b	Y = 777.940 – 8.2720x*	0.95
Canola	806.0 a	786.2 a	783.0 a	702.5 a	Y = 801.3050 + 2.8310 - 0.6070x2*	0.92
CV (%)	.... 3.03					

Means followed by different lowercases in the column (additive) are significantly different by Tukey's test (P<0.05). *significant at 0.05 level.

The inclusion of additives promoted a positive linear increase in the content of CP of the silage, observed for all used meals. The addition of 15% resulted in CP contents of 208.7; 164.0; 232.5 and 170.0 g/kg for the meals of cotton, sunflower, soybean and canola, increasing 129; 86; 154 and 92 percentage units when compared to the non-addition. This can be explained by two reasons: the first related to the higher content of CP of the meals in relation to piatã grass, so their addition improve the silage quality; the second, owing the higher contents of DM of the meals at the level of 15%, restricting the *Clostridium* activity, preserving thus the protein fraction of the forage (Aguiar et al., 2001).

At levels 5, 10 and 15% the sunflower and canola meals had the lowest contents of CP, differing from soybean and cotton meals which promoted an expressive increase in CP content of the silage (Table 3). This result is correlated with the CP content of the meals of soybean (420.4 g/kg) and cotton (417.0 g/kg) in relation to the other meals. Zanine et al. (2010) observed the importance of working with additives with high CP content to improve the silage and provide a better quality food.

Likewise, Gonçalves et al. (2007) achieved increases in the CP content with the use of cashew apple in the silage of elephant-grass silage and *Brachiaria decumbens* and Paziani et al. (2006), with inclusion of millet meal in tanzania-grass silage.

Moreover, the additives were effective to increase the EE content of the silage regardless the used meal, promoting a positive linear effect (Table 3). This increase is caused by the higher content of EE of the meals compared to the piatã grass (Table 1).

At the levels 0 and 10%, the contents were similar between the meals. But at the level of 5%, only the cotton meal was different from the others, with lower values of EE. And at the level of 15%, the meals of canola and sunflower presented the greatest contents, compared to cotton and soybean. Importantly, the EE content of meals from agro-industries usually depends on the species used, seed quality, and method used to extract the oil, given the great variation between the analyzed samples (Oliveira et al., 2011).

Moreover, regardless of additive and inclusion level, the EE content did not exceed 60-70 g/kg in the DM. According to NRC (2001), the total dietary fat should not exceed these values, since it may reduce the ruminal fermentation, fiber digestibility, and passage rate.

Rezende et al. (2002) studied the nutritional value of the elephant-grass silage added with sunflower and verified an EE content of 74.9 g/kg in the silage added with 50% sunflower, which is explained by the participation of sunflower seed in the silage, with higher levels of inclusion.

Considering the mineral matter, there was a linear reduction for the meals of cotton, sunflower and soybean, with increasing levels in the silage. Nevertheless, for the canola meal, a quadratic reduction was observed, where the minimum point was estimated at the level of 14.5%.

When compared the additives within each level, the soybean and canola meals at 10% presented similar contents of mineral matter, being distinct from cotton and sunflower. But at the level of 15%, only the cotton meal was different from the others. For all meals, the level zero presented the highest contents of mineral matter due to the increased potential for losses in these treatments with inadequate fermentation, with losses of organic matter, increasing the relative participation of ash (mineral matter) in the DM (Ashbell, 1995).

Oliveira et al. (2011) evaluated the inclusion of palm cake in the ensiling of massai-grass and verified a negative linear trend of the mineral matter as a function of its levels in the silage, and for each 1% inclusion of cake, there was a reduction of 0.137 percentage units of mineral matter.

With the addition of the examined levels, there was a linear decrease in the content of TCH with increasing levels of cotton, sunflower and soybean meals in the silage, and a quadratic reduction for the canola meal, estimating a maximum value of 702 g/kg for the level of 14.6%. This decrease is related to the higher contents of CP and EE at the 15% level. Sniffer et al. (1992) registered that high contents of CP and EE can reduce the estimation of TCH content, due to the high content of these fractions.

At zero level, the content of TCH was similar between the meals. At the levels of 5, 10 and 15%, the meals of cotton, sunflower and soybean had similar results, differing from canola, which presented the highest content of TCH. Carvalho et al. (2007) evaluating the silage of elephant-grass with cocoa meal, observed a linear reduction in the content of TCG, with a decrease of 0.22 percentage units for every unit of added meal. The same was observed by Andrade et al. (2010), who analyzed the elephant-grass silage and verified a reduction of 9.21% in the content of TCH of cocoa meal in relation to other additives.

For the NDF, a linear reduction was observed for the meals of cotton, sunflower and soybean, with increasing levels of additives. However, the canola meal led to a quadratic decrease, reaching the minimum at the level of 14.3% (Table 4). The additives inclusion is important because the piatã grass has a high content of NDF, and the partial replacement by the meals allowed the fiber dilution, promoting a reduction in the NDF content in the silage.

Table 4. Fibrous fractions of piatã grass ensiled with different levels of additives.

Additives	**Additive levels**				**Equation**	R^2
	0	**5**	**10**	**15**		
	Content of NDF (g/kg)					
Cotton	713.2 a	685.3c	634.0 b	584.5 b	$Y = 719.700 – 8.7607x^*$	0.98
Sunflower	711.0 a	699.5 bc	656.3 b	623.2 a	$Y = 718.300 – 6.1400x^*$	0.96

Soybean	710.5 a	695.0 bc	605.3 c	581.4 b	$Y = 719.475 – 9.5550x^{*}$	0.91
Canola	720.0 a	725.2 a	696.0 a	608.1 ab	$Y = 718.750 + 6.650x – 0.930x^{2*}$	0.99
CV (%)	.... 2.54					
Content of ACF (g/kg)						
Cotton	449.2 a	443.0 a	372.1 b	339.0 a	$Y = 460.900 – 8.0200x^{*}$	0.91
Sunflower	446.3 a	422.4 a	364.0 b	328.5 a	$Y = 451.800 – 8.2400x^{*}$	0.97
Soybean	444.0 a	387.2 b	357.1 b	323.2 a	$Y = 436.700 – 7.8600x^{*}$	0.97
Canola	443.1 a	437.0 a	404.5 a	330.4 a	$Y = 442.300 + 2.760x - 0.6800x^{2*}$	0.99
CV (%)	.... 4.17					
Content of Lignin (g/kg)						
Cotton	53.0 a	48.2 a	43.1 a	39.0 a	$Y = 52.800 – 0.9400x^{2*}$	0.99
Sunflower	52.3 a	47.0 a	42.2 a	33.4 b	$Y = 52.800 – 1.2400x^{2*}$	0.97
Soybean	54.0 a	51.3 a	44.2 a	35.0 b	$Y = 55.600 – 1.2800x^{*}$	0.95
Canola	51.2 a	48.1 a	43.0 a	34.5 b	$Y = 51.600 – 1.0800x^{*}$	0.95
CV (%)	.... 5.98					
Content of Cellulose (g/kg)						
Cotton	395.2 a	381.5 a	329.2 b	299.3 a	$Y = 399.500 – 3.100x – 0.2600x^{2*}$	0.93
Sunflower	393.0 a	374.2 a	321.1 b	294.2 a	$Y = 397.500 – 7.500x + 0.0200x^{*}$	0.94
Soybean	394.5 a	335.1 b	320.2 b	313.0 a	$Y = 392.200 – 12.960x + 0.520x^{2*}$	0.98
Canola	392.0 a	389.1 a	360.3 a	293.0 a	$Y = 391.400 + 3.080x - 0.6400x^{2*}$	0.99
CV (%)	.... 4.63					
Content of Hemicellulose (g/kg)						
Cotton	283.4 b	261.1 b	245.2 ab	241.0 bc	$Y = 278.800 – 2.8400x^{*}$	0.92
Sunflower	295.0 a	282.3 a	277.0 a	263.1 a	$Y = 294.400 – 2.0200x^{*}$	0.97
Soybean	302.1 a	263.0 b	236.1 b	228.3 c	$Y = 294.600 – 4.9800x^{*}$	0.92
Canola	292.2 a	287.5 a	288.3 a	277.0 ab	ns	
CV (%)	.... 7.44					

Means followed by different lowercases in the column (additive) are significantly different by Tukey's test ($P<0.05$). *significant at 0.05 level, ns = non-significant at 0.05 level.

At the level zero, the content was similar between the meals. The levels of 5 and 10% indicated the canola meal with the highest content of NDF, differing from the other meals. And at the 15% level, the meals of soybean and cotton presented the lowest content of NDF, proving to be more effective to reduce this fraction, which has more indigestible components. These results are assigned to the lowest content of fiber in the soybean and cotton meals (Table 1) compared with sunflower and canola. Lima et al. (2002) reported that the content of NDF is important to improve the nutritional value of the forage, being an important parameter to define the forage quality, because the most fibrous food takes up space for longer time and limits the intake rate.

The decrease in the content of NDF also was found by Ribeiro et al. (2008), which added wheat meal in the silage of elephant-grass, and by Costa et al. (2011) when added millet meal in the silage of marandu, xaraés and piatã grass.

The content of ADF was also reduced with the addition of the meals, with a linear decrease for the increasing levels of cotton, sunflower and soybean in the silage, and a quadratic reduction with the minimum point at the level of 14.0% for the canola meal (Table 4). These results are owed to the low content of fiber of the meals (Table 1). At the level 5%, the lowest content was obtained for the soybean meal. For the level 10%, only the canola meal was different from the other with the highest content. And at the level zero and 15%, the ADF content was similar between the meals.

The ADF content observed in the silage added with 10 and 15% of meals was within the adequate range, once Nussio et al. (1998) reported that forages with ADF content around 400 g/kg or more have low intake and digestibility.

When added the palm cake in ensiling massai grass, Oliveira et al. (2011) found a linear reduction in the NDF and ADF content of the silage, and for each 1% of palm cake added, a reduction of 0.099 and 0.101 percentage units was achieved for the NDF and ADF.

Regarding the lignin, the Table 4 shows that all the additives promoted a linear effect, with a reduction in the content of lignin with increased levels of additives, most expressive at the level of 15%, due to the dilution effect of this fraction. These results are relevant to improve the silage quality, by providing a food with low lignin content, which is important because it is not a carbohydrate but an amorphous phenylpropanoid polymer with structural function, indigestible, and inhibits the plant digestibility (Maranhão et al., 2009). Van Soest (1994) stated that lignin has a great negative influence on the degradation rate and effective degradability of cell wall of the forages, besides having silica and cutin.

At levels zero, 5 and 10% the lignin content was similar between the meals. But at the level 15%, the influence of the additives was evidenced, and the cotton meal presented the highest content. This is due to the higher content of lignin in the cotton meal (43.9 g/kg).

Analyzing the tanzania grass, Ribeiro et al. (2008) observed that the lignin content decreased with the inclusion of wheat bran, pointing out that this additive made more digestible the silage. This result can be explained by the recalcitrance ability of the lignin, which is minimized, allowing a better use of the fiber by the microorganisms.

For the content of cellulose, the meals inclusion promoted a quadratic decrease with minimum point at the levels of 14.1; 13.9, 14.5 and 14.7%, for the meals of cotton, sunflower, soybean and canola, respectively. Van Soest (1994) explained that cellulose is the most important structural portion of the cell wall; its availability varies from indigestible to completely digestible, depending on the lignification degree. The cellulose content remained between 200 and 400 g/kg DM, as recommended by Van Soest et al. (1994) for tropical forage.

Comparing the additives within each level, at the levels zero and 15%, the cellulose content was similar between the meals (Table 4). Meanwhile, for the 5% level, the soybean level reached the lowest level and at the level of 10%, only the canola meal was different from the other, with the highest cellulose content. Bernardino et al. (2005) evaluated the elephant-grass silage with different levels of coffee husk, and recorded an average content of cellulose of 385.0 g/kg, showing that the addition of coffee husk had no influence on the cellulose content.

The content of hemicellulose also had reduced with increasing levels of additives, with a linear decrease for the cotton, sunflower and soybean meals. This can be explained by its hydrolysis, which according to McDonald et al. (1991) is caused by several factors, such as: enzymatic activity of the hemicellulose in the forage and produced by the bacteria, and the acid hydrolysis owed the organic acids produced during the fermentation. In agreement with Hunt et al. (1993) the hemicellulose seems to be the main substrate for fermentation, after the use of soluble carbohydrates, with a possible degradation of 50% of the total in the original organic matter.

At the level zero, the content of hemicellulose was similar between the meals. But at the levels 5, 10 and 15%, lower contents were detected in the cotton and soybean meals, by presenting the lowest contents of hemicellulose (Table 1), differing from sunflower and canola. Henderson (1993) reported that protein, amino acid, and organic acid contribute to the production of acids; however the hemicellulose fermentation is the major additional source of substrate.

Even with the variation between the levels within each additive, the present study showed that the content of hemicellulose obtained for the additives met the recommended by McDonald et al. (1991) for grasses, ranging from 100 to 300 g/kg DM.

Conclusion

The oleaginous meals from biodiesel industry can be considered as a good source of additives for ensiling the piatã grass, by improving the qualitative, nutritional and fermentative characteristics of the piatã grass silage. It is recommended the addition of 15% of the meals, since this level provided the best quality silage. The soybean meal was the most effective to improve the silage quality, when compared with the other additives.

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CAPITULO 2

Piatã grass ensiled with different levels of meals from biodiesel industry: protein fractionation and digestibility

ABSTRACT: The goal of this study was to evaluate the protein fractionation and the in vitro digestibility of dry matter of piatã grass ensiled with different meals from biodiesesl industry. The experiment was developed in the Federal Institute of Goiás State, Campus Rio Verde, with a completely randomized experimental design, four replications, in a 4 x 4 factorial scheme, with four oleaginous meals (cotton, sunflower, soybean and canola) and four levels of inclusion (0, 5, 10 and 15%). The results demonstrated the considerable improvement in the protein fractions and in vitro digestibility of dry matter by using the meals from biodiesel industry. The inclusion of 15% of the meals provided the best quality forage. Among the meals, the one of soybean was the most effective, because presented the greatest fractions A and B1 and the lowest fraction C, besides the best digestibility, compared with other additives.

KEY WORDS: additive, *brachiaria brizantha*, silage

RESUMO: Desenvolveu-se esse estudo com objetivo de avaliar o fracionamento de proteínas e digestibilidade *in vitro* da matéria seca da Silagem de capim-piatã ensilado com diferentes farelos da indústria de biodiesel. O experimento foi conduzido no Instituto Federal Goiano, Campus Rio Verde. O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições, em esquema fatorial 4 x 4, sendo quatro farelos oleaginosos (algodão, girassol, soja e canola) e quatros níveis de adição (0, 5, 10 e 15%). Os resultados demonstraram que os farelos da indústria do biodiesel, apresentam boas fontes de aditivos para a ensilagem do capim-piatã, por trazer melhorias consideráveis nas frações proteínas e digestibilidade *in vitro* da matéria seca. Diante disso, recomenda-se a adição do nível de 15% dos farelos, por proporcionar melhor qualidade da silagem. Dentre os farelos utilizados o de soja mostrou mais eficiente por apresentar maiores frações A e B1 e menor fração C, além da melhor digestibilidade, quando comparados com os outros aditivos.

Palavras-chave: aditivo, *Brachiaria brizantha*, ensilagem.

Introduction

The difficulty to achieve good quality foods at certain year seasons in some localities is the main reason that leads the farmers to produce silage (Carvalho et al., 2008). Under suitable climatic conditions, the piatã grass besides used for grazing, also has appropriate nutritional characteristics for making silage (Costa et al., 2011). However, due to intrinsic characteristics at the ideal stage for cutting (moisture content, soluble carbohydrates, and low buffering capacity), tropical grasses silage may undergo losses of energy and dry matter ranging from 7 to 40%, due to secondary fermentation, produced effluents and aerobic deterioration (Santos et al., 2008; Ribeiro et al., 2009).

In this way, the losses of silage must be reduced, and one way for this is by including additives, in order to increase the dry matter content and provide better fermentation. Thus, the incorporation of new nutrient sources becomes usual and some byproducts, like meals, have been evaluated (Bernardes et al., 2005; Santos et al., 2010; Costa et al., 2011 and Oliveira et al., 2011), enhancing the silage quality.

In order to evaluate the final silage, new systems and methods for assessing foods for ruminant are being used to maximize the use of nutrients by the animals. The *Cornell Net Carbohydrate and Protein System* (CNCPS) is a system that takes into account the dynamics of ruminal fermentation and potential loss of nitrogen, such as ammonia, in the evaluation of food (Sniffen et al., 1992), aiming to adjust the ruminal digestion of carbohydrate and protein to take full advantage of microbial production, reduce the loss of nitrogen, and estimate the ruminal loss of nutrient (Balsalobre et al., 2003).

Information on chemical-bromatological composition and food intake are valuable, but also is essential, the knowledge of the use of nutrients by the animal, achieved by studies on digestion and ruminal degradability, in order to express the nutritional value of a certain food, considering the variation between the animals. Among the techniques adopted to evaluate the ruminal degradation, the in vitro method represents the digestion process in the rumen, abomasum or intestine to quantitatively estimate the rate and degree of digestion, similarly to what in vivo (Berchielli et al., 2006).

In this context, this study evaluated the protein fractionation and in vitro digestibility of dry matter of piatã grass ensiled with different levels of meals from biodiesel industry.

Material and Methods

The experiment was performed in the Federal Institute of Goiás State, Campus Rio Verde, at 748 m altitude, 17° 48' south latitude and 50^{o} 55' west longitude, from September 2010 to July 2011. The area of pasture used to produce the silage had 180 m^2.

The soil was classified as distroferric Red Latosol (Oxisol), with 530 g/kg clay; 250 g/kg silt and 220 g/kg sand. The chemical characteristics of the soil at the layer 0-20 cm, before planting were: pH in water: 5.6; Ca: 4.04 $cmol_c/dm^3$; Mg: 2.0 $cmol_c/dm^3$; Al: 0.0 $cmol_c/dm^3$; Al+H: 6.6 $cmol_c/dm^3$; K: 65 mg/dm^3; CTC: 7.05 $cmol_c/dm^3$; P: 8.07 mg/dm^3; Cu: 3.7 mg/dm^3; Zn: 1.8 mg/dm^3; V: 48.4%; M.O: 35.6 g/kg.

The area was prepared with harrowing followed by leveling. By the planting of forage it was applied 80 kg/ha P_2O_5, using the super triple phosphate. Then, the piatã grass was broadcast seeded with 9 kg viable pure seeds per hectare. The standardization cut was held at 40 days after planting, and the topdressing included 80 kg/ha nitrogen and 40 kg/ha potassium, as urea and potassium chloride, respectively.

The experimental design was the completely randomized with four replications in a 4 x 4 factorial scheme, being four meals from biodiesel industry (cotton, sunflower, soybean, canola) and four levels of inclusion (0, 5, 10 and 15%). The meals were obtained from mechanical extraction of oil, where the amount applied was based on the natural material of piatã grass.

For the ensilaging process, the piatã grass was harvested at 45 days after maintenance fertilization, at 20 cm from ground level, using backpack crush cutter. Afterwards, the forage was minced into 10-30mm-particles, with a stationary shredder, and ground. Then, the material was homogenized with the meals, according to the different levels determined, and stored in PVC experimental silo, with 10cm diameter and 40cm length.

The silage was compacted with iron pendulum and the silos were sealed with PVC caps and adhesive tape to preclude the entry of air. Immediately after, they were stored at room temperature and protected from rain and sunlight.

After 60 days of fermentation, the silos were opened, discarding the top and bottom portion of each. The central portion was homogenized and placed into plastic trays. About 1 kg was weighed and taken to a forced air oven at 55ºC for 96 hours to determine the the pre-drying matter. Later, the samples were ground in a Willey type mill, with 1mm-sieve, to be analyzed.

Non-protein nitrogen (NPN), neutral and acid detergent insoluble nitrogen (NDIN and ADIN) were determined according to Licitra et al. (1996), and the soluble nitrogen (SN), according to Krishnamoorthy et al. (1982).

The protein fractionation was calculated by the CNCPS system (Sniffen et al., 1992). The protein was analyzed and calculated for the five fractions, A, B1, B2, B3 and C. The fraction A, made up by NPN compounds, was determined by the difference between total N and trichloroacetic acid (TCA) insoluble nitrogen with the formula: A (%Nt) = Nt - N1 / Nt x 100, where Nt = total nitrogen of the sample, and N1 = content of trichloroacetic acid insoluble nitrogen. The fraction B1 refers to soluble protein, rapidly degraded in the rumen, obtained by the difference between the borate phosphate buffer (TBF) insoluble nitrogen minus the NPN, by the formula: B1 (%Nt) = N1 - N2 / Nt x 100, where: N2 = borate phosphate buffer insoluble nitrogen. The fractions B2 and B3 consist of insoluble protein with intermediate-slow degradation rate in rumen, determined by the difference between the borate phosphate buffer insoluble nitrogen and NDIN, the NDIN minus the ADIN, respectively. The value of B2 is achieved with B2 (%Nt) = N2 - NIDN / Nt x 100 and the fraction B3, with B3 (% Nt) = NDIN - ADIN / Nt x 100. The fraction C is formed by insoluble protein indigestible in the rumen and intestine, and was determined by the content of residual nitrogen of the sample after treated with acid detergent and expressed in percentage of Nt of the sample. The in vitro digestibility of dry matter (IVDDM) was determined according to Tilley and Terry (1963), with two 48 h-incubation cycles.

Data were subjected to analysis of variance and regression analysis, and the mean values were compared by the Tukey's test at 5%, using the statistical software SISVAR 4.6 (Ferreira, 2000).

Results and Discussion

The fractions A, B1, B2, B3 and C were significantly influenced ($P<0.05$) by the additives, inclusion levels and interaction between these factors (Table 1), as also observed for the in vitro digestibility of dry matter (Table 2).

The fraction A or non-protein nitrogen (NPN) increased linearly with increased levels of meals in the silage. For all the meals, the level of 15% was the most effective in increasing the fraction A. This is an outstanding result because this soluble fraction has rapid ruminal degradation owed the higher content of CP in the meals, improving thus the degradation rate. According to Russell et al. (1992), non-protein nitrogen sources are essential for the proper functioning of the rumen, since ruminal microorganisms fermenting structural carbohydrates use ammonia as a nitrogen source.

At level zero and 5%, the fraction A was similar between the additives. At the level 10 and 15%, the soybean meal had the highest values of fraction A, differing from cotton, sunflower and canola meals, with similar fractions. Therefore, the higher the increase in fraction A, the greater the need for carbohydrates of rapid degradation for a suitable synchrony of fermentation of carbohydrate and protein in the rumen (Russell et al., 1992).

Andrade et al. (2010) evaluated the silage of elephant-grass with cassava and cocoa meals and coffe husk, and observed a downward linear trend as increased the additive levels. According to these authors this was due to the low content of dry matter (187.0 g/kg) interfering with the availability of degradable protein.

Table 1. Protein fractionation of piatã grass ensiled with different levels of additives.

Additive	**Level**				**Equation**	R^2
	0	**5**	**10**	**15**		
	Fraction A (g/100 g)					
Cotton	32.48 a	36.32 a	42.79 b	51.40 b	Y = 31.2630 + 1.2646x*	0.97
Sunflower	31.88 a	36.49 a	41.71 b	50.99 b	Y = 30.8850 + 1.2510x*	0.97
Soybean	33.68 a	35.43 a	43.27 a	54.59 a	Y = 31.1570 + 1.4114x*	0.91
Canola	32.82 a	37.85 a	41.35 b	50.06 b	Y = 32.2370 + 1.1044x*	0.96
CV (%)	.... 3.23					
	Fraction B1 (g/100 g)					
Cotton	7.38 b	8.12 a	9.98 b	11.12 b	Y = 7.1880 + 0.2616x*	0.97
Sunflower	8.48 ab	8.81 a	10.38 b	11.61 b	Y = 8.1760 + 0.2192x*	0.94
Soybean	8.57 ab	9.07 a	11.07 a	12.00 a	Y = 8.3340 + 0.2458x*	0.95
Canola	8.89 a	8.80 a	9.72 b	11.13 b	Y = 8.8640 - 0.0722x + 0.0150x2*	0.99
CV (%)	.... 8.23					
	Fraction B2 (g/100 g)					
Cotton	10.80 a	12.55 b	13.56 b	14.69 a	Y = 10.9980 + 0.2536x*	0.98
Sunflower	11.87 a	14.87 a	16.47 a	15.94 a	Y = 7.2070 + 1.8284x - 0.0840x2*	0.98
Soybean	12.07 a	13.42 a	14.96 ab	14.97 a	Y =11.9840 + 0.4058x - 0.0134x2*	0.97
Canola	11.16 a	12.99b	15.57 a	14.62 a	Y= 10.9460 + 0.6762x - 0.0278x2*	0.91
CV (%)	.... 8.07					
	Fraction B3 (g/100 g)					
Cotton	16.26 a	15.36 a	10.26 ab	7.33 a	Y = 17.0860 - 0.6378x*	0.94
Sunflower	15.52 a	13.25 a	10.17 c	7.90 a	Y = 15.6010 - 0.5188x2*	0.99
Soybean	15.04 a	14.81 a	10.23 ab	7.29 a	Y = 16.0170 - 0.5566x*	0.91
Canola	16.68 a	14.47 a	12.49 a	7.55 a	Y = 17.2030 - 0.5874x*	0.94
CV (%)	.... 9.93					
	Fraction C (g/100 g)					
Cotton	33.06 a	27.63 a	23.41 a	15.46 ab	Y = 33.4430 - 1.1404x2*	0.98
Sunflower	32.24 a	26.58 b	21.23 b	13.52 b	Y = 32.6190 - 1.2302x2*	0.99
Soybean	30.61 b	27.26 a	20.44 b	11.11 c	Y = 32.1530 - 1.3064x2*	0.95
Canola	30.45 b	25.86 b	20.85 b	16.61 c	Y = 33.4430 - 1.1404x2*	1.00
CV (%)	.... 5.52					

Means followed by different letters, lower case in the columns (additive), are significantly different by Tukey's test ($P<0.05$). *significant at 0.05 level.

Considering the fraction B1, a linear increase was observed for the meals of cotton, sunflower and soybean according to the addition in the silage, and a quadratic increase for canola meal, estimated the minimum point at the level of 14.3% (Table 1). The highest fractions B1 were obtained at 15% level for all meals, with increase of 3.74; 3.13; 3.43 and 2.24 percentage units compared to level zero, for cotton, sunflower, soybean and canola, respectively. Importantly, the contribution of 15% in the ensiling process increased the fraction B1, favoring the degradation in the rumen, since it can ensure a better synchrony of fermentation between carbohydrate and protein in the rumen, and consequently, favor the microbial growth, which results in better use of nutrients (Pereira et al., 2010). These results are relevant, once the fraction B1 is also considered the soluble fraction with rapid degradation in the rumen (Sniffen et al., 1992).

Comparing the additives, the fraction B1 was similar between the meals at the level of 5%. At zero level, a significant difference was found between the meals of cotton and canola. And at levels 10 and 15%, the soybean meal had the highest value of the fraction B1. This result is correlated with the high content of CP of the soybean meal (42.0%).

Some authors reported the deficiency of the fraction B1 in the protein of tropical forage (Russell et al., 1992; Sniffen et al., 1992), with values below 10 g/100 g of total crude protein (Balsalobre et al., 2003). Nevertheless, with the addition of 15% of both meals, the fraction B1 was enhanced, with values higher than 11.12 g/100 g.

On the silage of tifton-85, Pereira et al. (2007) verified a value of 2.97 g/100 g for the fraction B1 for the control silage, while when added the waste from corn processing and corn meal, the fractions were 2.11 and 1.91 g/100 g, respectively.

The fraction B1 + B2, by having a rapid degradation rate in the rumen in relation the fraction B3, tends to be extensively degraded in the rumen, contributing to meeting the requirements of nitrogen of the ruminal microorganism, but the rapid ruminal proteolysis of these fractions may lead to the accumulation of peptides and allow their loss to the intestine, once the use of peptides is limiting for protein degradation (Sniffen et al., 1992).

There was a linear increase of the fraction B2 for the cotton meal with the addition of the meals in the silage, being this increase more expressive at the 15% level. But, for the meals of sunflower, soybean, and canola, a quadratic increase was found with the maximum point at the levels of 17.2; 15.05 and 15.06%, respectively.

Among the meals within each level, observed in the Table 1, at the level zero and 15%, the fractions B2 were similar between the meals. At the 5% level, the sunflower and canola meals presented greater fractions B2 and at the level of 10%, only the cotton meal was different from the sunflower and canola meals.

In this way, Andrade et al. (2010) studied the elephant-grass ensiled with different additives and verified that when added 30% of the levels, the silage with cassava meal presented the highest fraction B1 + B2 (45.6 g/100 g), whereas the coffee husk had intermediate values (33.3 g/100 g) and the cocoa meal achieved lower fractions B1 + B2 (30.0 g/100 g).

The fraction B3 has a very slow degradation rate, once it is associated with the cell wall. This fraction is represented by extensive cell-wall-bound proteins, thus slowly degraded, and mainly digested in the guts (Balsalobre et al., 2003).

The inclusion of the meals promoted a linear reduction of the fraction B3 for all meals, whose reductions were estimated at 8.93; 7.62; 7.75 and 9.13 percentage units, when comparing the level zero with 15%, for the meals of cotton, sunflower, soybean and canola, respectively. These results are assigned to the lowest content of CP of piatã grass in relation to the meals, thus, higher fractions B3 are achieved in the control, with a slower degradation.

As the fraction B3 is represented by cell-wall-bound proteins with slow degradation rate and mainly digested in the guts (Cabral et al., 2004), it seems that without the addition of the meals in the piatã grass silage, the ruminal non-degraded protein increases, once greater values of B3 and sharp reduction of the fraction A were observed in the control silage.

In relation to the meals within each level, the fraction B3 was similar between the meals at the level zero, 5 and 15%. Only the level 10% was influenced, presenting a greater fraction B3 for the canola meal.

Pires et al. (2009) evaluated the fractionation of proteins of the elephant-grass silage, and verified fractions B3 of 10.7; 11.6; 21.5 and 19.5 g/100 g for the control, with addition of coffee husk, cocoa meal, and cassava meal, respectively.

The fraction C has linearly reduced for all used meals, as increased the levels of additives in the ensiling process, with a reduction of 17.6; 18.7; 19.5 and 13.8 percentage units, comparing the level zero with the level 15%, for the meals of cotton, sunflower, soybean and canola, respectively. This is a remarkable result because the fraction C corresponds to unavailable nitrogen and consists of protein and nitrogen compounds associated with lignin (Sniffen et al., 1992; Van Soest, 1994). Krishnamoorthy et al. (1983) reported that this fraction is not degraded by bacteria in the rumen and does not provide amino acids for the post-ruminal digestion. This shows that an expressive protein portion from silages is not used for microbial growth or even as a true protein source in the post-ruminal digestive tract.

Our findings are satisfactory considering the silage added with additives, since according to Sniffen et al. (1992), the increase in the fraction C is complicated by the formation of Maillard products caused by the heating inside the silo by the undesirable fermentation owing the high moisture content of forage. In this case, the decreased fraction C when added the levels, mainly at the 15% level, can be ascribed to the meals' quality, being considered good additive for ensiling process.

Carvalho et al. (2008) examined the protein fractionation of the elephant-grass ensiled with cocoa meal, and observed a quadratic behavior for the fraction C as a function of the inclusion levels, estimating the maximum value of 33.0 g/100 g for the level of 19.6%.

Regarding the meals within each level, at the level zero, the cotton and sunflower meals presented similar values of the fraction C, differing from soybean and canola meals. At 5% level, the lowest fractions were detected for the sunflower and canola meals. At 10%, the cotton meal had the highest fraction, different from the other meals. And at the 15% level, the soybean meal was the most effective in reducing the fraction C, with a value of 11.1 g/100 g.

By assessing the silage of elephant-grass with cassava meal, coffee husk and cocoa meal, Andrade et al., (2010) observed a downward linear effect of including the cassava meal to the silage, with a reduction estimated at 0.13 percentage unit for every 1% of cassava meal added. Higher results were found in the present study, with values of fraction C varying between 11.11 and 16.61 g/100 g when added 15% of soybean and canola meals, respectively.

The in vitro digestibility of dry matter (IVDDM) increased linearly for the meals of cotton and canola with increased levels of the meals in the ensiling process, and a quadratic increase, with the maximum point at the level 14.0% for the sunflower, and 13.7% for the soybean meal. Comparing the level zero with the 15% level, there was increase of 0.06; 0.091; 0.159 and 0.085 percentage units for the cotton, sunflower, soybean and canola meals, respectively. This increased digestibility is probably associated with the changes in the chemical composition of the fraction with a reduced content of NDF and ADF and of hemicellulose, which certainly make available the carbohydrates readily digestible for the ruminal microorganisms (Fernandes et al., 2002).

Rezende et al. (2008) studied the elephant-grass silage added with potato scrapings and registered a linear increasing response (0.582; 0.622; 0.662; 0.702 e 0.742 kg/kg) for the IVDDM in relation to the inclusion of the levels 0, 7, 14, 21 and 28%, respectively. Similar results were found by Rodrigues et al. (2007) with elephant-grass ensiled with citrus pulp, when they verified a linear increase in the IVDDM with increased addition of pulp in the silage.

Table 2. In vitro digestibility of DM (kg/kg) of the piatã grass ensiled with different levels of additives.

Additives	**Levels**				**Equation**	R^2
	0	**5**	**10**	**15**		
Cotton	0.540 a	0.555 d	0.572 d	0.600 c	$Y = 0.5372 + 0.0039x^{*}$	0.97
Sunflower	0.537 a	0.607 b	0.620 b	0.628 b	$Y = 0.5396 + 0.0150 - 0.0006x^{2*}$	0.97
Soybean	0.543 a	0.680 a	0.695 a	0.702 a	$Y = 0.5487 + 0.0293 - 0.0013x^{2*}$	0.96
Canola	0.535 a	0.587 c	0.598 c	0.620 b	$Y = 0.5451 + 0.0053x^{*}$	0.90
CV (%)	.... 1.52					

Means followed by different letters, lower case in the columns (additive), are significantly different by Tukey's test (P<0.05). *significant at 0.05 level.

In relation to the additives within each level, at the level zero, the digestibility was similar between the meals. At the levels 5 and 10%, there was a significant effect for all the meals, with the lowest digestibility found for the cotton meal. And at 15%, the soybean meal had the highest digestibility, with increase of 17.0; 11.7 and 13.2% in relation to the meals of cotton, sunflower and canola, respectively, proving to be an excellent option of additive in tropical grass silage, by containing high protein and energy content. According to Jobim et al. (2010) the soybean can represent an option to be employed in the ensiling process, besides having an unquestionable nutritional value.

Conclusion

The meals from biodiesel industry are good sources of additives for the piatã grass silage, by bringing about considerable improvements in protein fractions and in vitro digestibility of dry matter. The inclusion of 15% of the meals provided the best quality forage, being thus the recommended level. Among the meals, the one of soybean was the most effective, because presented the greatest fractions A and B1 and the lowest fraction C, besides the best digestibility, compared with other additives.

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CONCLUSÃO GERAL

Os farelos oleaginosos da indústria de biodiesel podem ser considerados bons aditivos para a silagem de capim-piatã, por melhorar o valor nutritivo. No entanto, dentre os farelos utilizados o de soja se destacou por promover melhores características fermentativas, bromatológicas, fração proteína e digestibilidade *in vitro* da matéria seca.

Para quaisquer farelos estudados, o nível de 15% proporciona uma silagem de boa qualidade, mostrando um ajuste quadrático na grande maioria dos atributos analisados.