RICARDO GALBIATTI SANDOVAL NOGUEIRA

Enteric and feces methane emissions, ruminal fermentative parameters and feeding behavior of cows fed cottonseed and vitamin E.

Pirassununga

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Tese apresentada ao Programa de Pós Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências.

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Orientador:

Prof. Dr. Paulo Henrique Mazza Rodrigues.

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Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que o Projeto intitulado "Quantificação das emissões de metano entérico e dos dejetos, excreção de nutrientes e avaliação nutricional em bovinos alimentados com ou sem caroço de algodão: possibilidades de mitigação", protocolado sob o nº 3009/2013, utilizando 6 (seis) bovinos, sob a responsabilidade do(a) Prof. Dr. Paulo Henrique Mazza Rodrigues, foi aprovado em reunião de 26/6/2013 e está de acordo com os princípios éticos de experimentação animal da Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo.

We certify that the Research "Quantification of methane emissions from enteric and manure, nutrient excretion and nutritional evaluation in cattle fed with or without cottonseed: mitigation possibilities", protocol number 3009/2013, utilizing 6 (six) cattle, under the responsibility Prof. Dr. Paulo Henrique Mazza Rodrigues, was approved in the meeting of day 6/26/2013 and agree with Ethical Principles in Animal Research adopted by Ethic Committee in the Use of Animals of the School of Veterinary Medicine and Animal Science of University of São Paulo.

São Paulo, 27 de junho de 2013.

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RESUMO

NOGUEIRA, R. G. S. **Emissões de metano entérico e das fezes, variaveis fermentativos ruminais e comportamento ingestivo de bovinos alimentados com caroço de algodão e vitamina E** (Enteric and manure methane emissions, fermentative ruminal and behavioral parameters of cattle fed cottonseed and vitamin E). 2017. 97 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

A problemática das emissões de gases de efeito estufa atribuída à produção de bovinos e melhorias na produtividade desses animais vem crescendo e se tornando cada vez mais importante. Bovinos emitem metano como parte do seu processo digestivo, e isto representa perda de energia para o animal. A decomposição das fezes gera metano, este pode ser recuperado por biodigestores e transformado em diferentes tipos de energia. Assim, objetivou-se quantificar o potencial de produção do metano entérico e da decomposição anaeróbia das fezes, bem como avaliar parâmetros ruminais e comportamentais de bovinos alimentados com caroço de algodão e vitamina E. Foram utilizadas seis vacas fistuladas não gestantes e não lactantes (876 kg±16). Os tratamentos foram: 1) Controle: dieta basal; 2) CA: dieta basal mais 30% de caroço de algodão; 3) CAVitE: dieta basal mais 30% de caroço de algodão mais 500 UI vitamina E. O delineamento experimental utilizado foi o quadrado latino. Os resultados foram comparados por contrastes ortogonais e foram considerados significantes valores de $P \leq 0,05$. Não foram verificadas diferenças para o consumo de matéria seca (MS), bem como digestibilidade da MS e da fibra em detergente neutro (FDN). Os animais suplementados com caroço de algodão passaram maior tempo comendo e ruminando e menor tempo em ócio. Houve redução na concentração e produção de acetato, butirato e da relação acetato:propionato dos animais que receberam caroço de algodão comparado ao controle. A inclusão do caroço de algodão provocou mitigação das emissões de metano entérico. Houve alteração nas características dos substratos utilizados para abastecer os biodigestores. No entanto, não foram verificadas diferenças para a produção total de biogás, rendimento de metano e capacidade dos biodigestores em recuperar a energia das fezes na forma de metano. A inclusão de 30% caroço de algodão pode ser utilizada como estratégia para mitigar metano entérico, sem causar perdas no consumo, digestibilidade dos alimentos e na biodigestão anaeróbia das fezes. Além disso, sua inclusão promoveu alterações favoráveis no comportamento ingestivo, nos produtos da fermentação ruminal, bem como na partição de energia do trato gastrointestinal. A vitamina E quando utilizada como antioxidante não possui efeitos sobre a fermentação ruminal, comportamento ingestivo e biodigestão anaeróbia das fezes, assim sua inclusão não é indicada devido a ausência de resultados favoráveis a sua utilização.

Palavras-chave: Lipídeos. Antioxidante. Ruminantes. Biodigestão anaerobia.

ABSTRACT

NOGUEIRA, R. G. S. **Enteric and feces methane emissions, fermentative ruminal parameters and feeding behavior of cattle fed cottonseed and vitamin E** (Emissões de metano entérico e das fezes, parâmetros fermentativos ruminais e comportamento ingestivo de bovinos alimentados com caroço de algodão e vitamina E) 2017. 97 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

Problems about greenhouse gas emissions attributed to cattle production and improvements in the productivity of these animals has been growing and becoming increasingly important. Cattle releases methane as part of their digestive process, and this represents loss of energy for the animal. The decomposition of feces releases methane and it can be recovered by digester and transformed into different types of energy. Thus, aiming to quantify the potential production of enteric methane and anaerobic fecal decomposition, as well as to evaluate ruminal and behavioral parameters of cattle fed with cottonseed and vitamin E. Six cannulated cows (864±16 kg) were distributed in a replicate 3x3 Latin square. Treatments were: 1) control diet; 2) CS: basal diet plus 30% cottonseed and 3) CSVitE: basal diet plus 30% of cottonseed plus 500 UI of vitamin E. Results were compared through orthogonal contrast and values were considered significant when $P \leq 0,05$. No differences were observed for dry matter intake (DMI), as well as digestibility of DM and neutral detergent fiber (NDF). Animals supplemented with cottonseed spent more time eating and ruminating and less time in idles. Reduction in the concentration and production of acetate, butyrate and the acetate: propionate ratio was observed in animals fed cottonseed compared to the control. Enteric methane mitigation was observed for the cottonseed treatments compared to the control. Changes in the substrates characteristics used to load the digesters were observed. However, no differences were verified for the total biogas production, methane yield and capacity to recover the energy of the feces in the form of methane. Inclusion of 30% cottonseed can be used as a strategy to mitigate enteric methane, without causing losses in the DMI, nutrients digestibility and anaerobic digestion of feces. In addition, it promoted favorable changes in the ingestive behavior, ruminal fermentation products, as well as in the energy partition of the gastrointestinal tract. Vitamin E when is used as antioxidant had not effect on ruminal fermentation, feeding behavior and feces anaerobic digestion, thus the inclusion is not advised due absence of positive results.

Keywords: Lipids. Antioxidant. Ruminants. Anaerobic digestion.

SUMÁRIO

1. INTRODUCTION

While ruminants have an important role in providing high quality protein essential for the human diet, they are also an important source of greenhouse gas (GHG) emissions. Methane (CH_4), carbon dioxide (CO_2), nitrous oxide (N_2O) are the main GHGs emitted by livestock activity.

According to Beauchemin et al. (2008), the ruminant sector globally emits 35-40% of all methane produced by anthropogenic activities, with enteric fermentation accounting for 94% of all emissions. The remainder is produced by the decomposition of waste (FAO, 2013).

There is a concern of the cattle community not only with the environmental issue of enteric methane emissions and feces, but also with regard to productivity, since according to Buddle et al. (2011), from 5% to 9% of the raw energy of the diet is lost as methane.

Cattle are a source of enteric methane that can be manipulated because the production of this gas comes from ruminal fermentation, which is related to the animal category, DMI, type and digestibility of feed. There is possible to reduce enteric methane production by modifying ruminal fermentation, obtained by type and amount of carbohydrate included in the diet, by manipulating the ruminal microbiota with food additives and by adding lipids (Berchielli et al. 2012).

One of the strategies that has been prominente in the mitigation of methane emissions is the inclusion of lipids in the diet. The common sources used in lipid supplementation are vegetable oils and oilseeds (Machado Neto, 2011).

The use of vegetable oil has caused losses in animal productivity, mainly due to the reduction in the digestibility of some nutrients and dry matter intake (Berchielli et al. 2012). One of the alternatives found to minimize these problems has been the use of lipids protected from direct digestion in the rumen, which may be artificial or natural, such as oleaginous seeds (Freitas Junior, 2008).

Cottonseed is a co-product of the textile industry that is widely used in ruminant feed because it has a high concentration of protected oil, protein and fiber, which allows the replacement by concentrated feeds without losses ruminal

fermentation. Few feeds can gather these nutrients, in addition it has a high fiber degradability (Geron et al. 2011).

One of the consequences and concerns of using oilseeds in the diet of cattle is the increase in the concentration of unsaturated fatty acids, which increases the susceptibility to lipid peroxidation (Machado Neto, 2011).Unsaturated fatty acids oxidize easily, causing the development of unpleasant odor and taste, culminating in loss of the organoleptic characteristics of the meat (Pinto, 2010).

Therefore, strategies that may increase the lipid stability of meat are of great importance in the context of supplementation with lipid sources (Machado Neto, 2011). Antioxidants stabilize highly reactive free radicals, thus maintaining the structural and functional integrity of cells (Mendonça Junior, 2010).

Vitamin E is related to several functions in the body, and some of the most important are: i) inter- and intracellular antioxidant action; ii) inhibition of the natural peroxidation of unsaturated fatty acids in the lipid layers of the membrane, with the elimination of free radicals (Mendonça Junior, 2010).

Therefore, the main hypothesis this study is that inclusion of cottonseed causes reductions in the enteric methane emissions and changes in the ruminal fermentation products, as well as in the feces characteristics and anaerobic digestion. The secondary hypothesis is that the inclusion of vitamin E has effect on ruminal fermentation, and it has not effect on feeding behavior, feces characteristics and anaerobic digestion.

The objectives of this study are to evaluate the inclusion of 30% cottonseed and 500 IU of vitamin E on DMI, nutrients digestibility, ingestive behavior, ruminal fermentation products, as well as to verify characteristics of feces and this potential to produce methane in anaerobic conditions.

2. LITERATURE REVIEW

2.1. LIVESTOCK SECTOR

The livestock sector is large, twenty billion animals make use of 30% of the terrestrial land area for grazing, one-third of global cropland area is devoted to producing animal feed and 32% of freshwater is used to provide direct livelihood and economic benefits to at least 1,3 billion producers and retailers (Herrero et al. 2010; Thorton, 2010). As an economic activity, livestock contributes up to 50% of agricultural gross domestic product globally (FAO, 2009).

Global per capita consumption of livestock products has more than doubled in the past 40 years (FAO, 2009). Increasing human population, incomes and urbanization are projected to drive increases in the livestock consumption by 70% by 2050 (Geber et al. 2013). In response to these demand trends, the sector has intensified a significant increase production (FAO, 2006; Rosegrant et al. 2009), beef and milk production have more than doubled over the past 40 years (Thorton, 2010).

Previously observed rates (FAO, 2009; Rosegrant et al. 2009) most of the growth is projected to occur in the developing world. However, many parts of the developing world have high greenhouse gas (GHG) emissions from livestock, which are produced high intensities due to low productivity and large numbers of animals (for example, parts of Africa and Latin America) (Herrero et al. 2013).

The Food and Agriculture Organization of the United Nations (FAO) reported that GHG emissions from the livestock sector represent 18% of the global GHG emissions. Cattle production systems dominate the livestock sector's emissions with 64–78% depending on the study (FAO, 2013; Geber et al. 2013; Herrero et al. 2013). Taking an aggregate view of the sector, animal feed production accounts for about 45% of the sector's emissions, with about half of these emissions related to fertilization of feed crops and pastures (manure and fertilizer included) (Herrero et al. 2013). Enteric fermentation contributes to about 40% of total emissions, followed by manure storage and processing in which contributes to 10% of emissions (FAO, 2013).

2.2. ENTERIC FERMENTATION AND METHANE PRODUCTION

In ruminants, digestion of feed is a two-stage process (Mcallister et al. 2008; Hristov et al. 2013, Krause et al. 2013): (i) enzymatic degradation of feed sources in the rumen with the release of a range of monomers (sugars, amino acids, glycerol and fatty acids); and (ii) the fermentation of those compounds by rumen microbiota (bacteria, methanogenic Archaea, protozoa and fungi).

Rumen fermentation involves an oxidation process, generating reduced co-factors (NADH, NADPH, and FADH), which are then re-oxidized (NAD+, NADP and FAD+) by dehydrogenation reactions, releasing hydrogen in the rumen (McAllister and Newbold, 2008)

The pregastric fermentation of cellulose-rich feeds in the reticulo-rumen-omasal complex environment is intrinsically tightly regulated (redox potential of –300 to –350 mV; 38–42°C and pH 6–7). These conditions maintain ruminal microbial system functionality, but clearance of SCFA, H_2 and CO_2 must occur. Shor chain fatty acids (SCFA) are transported across the rumen and omasal walls and utilized by the animal, whereas CO_2 is released to the head space of the rumen and it lost through eructation or transported via circulation to the lungs, and then respired. The metabolic clearance of H_2 is either through SCFA production or, predominantly, conversion to CH_4. This latter process is facilitated by methanogenic Archaea. The major metabolic pathways are as follows by Knapp et al. (2013) and Jhonson and Jhonson, (1995):

Hydrogenotrophic: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
Methylotrophic: $CH_3OH + H_2 \rightarrow CH_4 + H_2O$
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$
$CH_3NH_2 + H_2 \rightarrow CH_4 + NH_3$
Aceticlastic: $CH_3COOH \rightarrow CO_2 + CH_4$ (minor in the rumen).

Methane production is therefore essential for obtaining a high-performing rumen ecosystem because H_2 accumulation, which could inhibit dehydrogenase activity in re-oxidation co-factors, is avoided. An efficient H_2 capture in the rumen contributes to increase in the rate of fermentation by the lack of inhibitory effect on the microbial degradation of organic material (Wolin, 1979; McAllister and Newbold, 2008).

Methanogenesis is an important part of the energy metabolism in ruminants and measuring this production is critical in understanding ruminant livestock productivity. For instance, increase in productivity can be achieved by reducing CH_4 through an increase capture of metabolic hydrogen H_2 into SCFA (Mitisumori et al. 2012). The loss of methane energy from ruminant is also a critical priority regarding feed energy utilization and animal productivity (Johnson and Johnson, 1995; Kurihara et al., 1999).

Emissions from enteric fermentation represent significant energy losses from feed intake. The CH_4 production per animal varies in 2 to 12% of the gross energy intake and it depends on the feed composition, feed quality, and production level (Johnson and Johnson, 1995). Under extreme circumstances values between 3 and 7% (Martin et al. 2008) are more realistic in intensive cattle production. It is possible that energy conserved from reduction on enteric methane emissions could be used in other metabolic processes, such as live-weight gain, milk and beef yield (Young et al. 2011).

Furthermore, the largest single contributor to livestock GHG emissions is enteric fermentation which represents between 32 and 40% of the total GHG emitted from the sector and have been the main focus for animal-based mitigation research (Smith et al. 2014). About 75 % of total CH_4 emissions from livestock come from cattle (Tubiello et al. 2013) and they are major contribution; 44% of global anthropogenic methane of GHG comes from methanogenesis during fermentation of feeds in the rumen (Gerber et al. 2013).

Therefore, methane mitigation is a priority for improving animal productivity and environmental sustainability (Beauchemin et al. 2011; Capper and Buaman, 2013).

2.3. METHANE MITIGATION

Extensive research in recent years has provided a number of practices viable for the enteric methane mitigation, such as alternative electron receptors, methane inhibitors, dietary lipids and increased animal productive efficiency (Histrov et al. 2013). Emissions intensities of livestock products may be reduced by manipulating farm management while improve animal production efficiency (Fergunson et al. 2007; Cruickshank et al. 2008; Alcock and Hegarty; 2011; Young et al. 2011). Numerous

studies have investigated the potential to decrease methane from enteric fermentation in ruminants using dietary strategies or dietary additives (Histrov et al. 2013).

The addition of fatty acids to ruminant diets increase dietary energy density, modifying rumen digestion and fermentation processes to mitigate methane production (Blaxter and Czerkawski, 1966; Machumuller et al. 2000; Lovett et al. 2003; Cieślak et al. 2006). Dietary supplementation with lipids reduces methane emissions through multiple mechanisms: i) reduction of fermentable organic matter (lipids are not a source of energy for rumen bacteria); ii) reduction of methanogenic activity due to the presence of medium-chain fatty acids; iii) toxic effects on cellulolytic bacteria (Nagajara et al. 1997) and protozoa (Doreau and Ferlay, 1995) due to the effect of long chain fatty acids and. Toxic effects of long chain fatty acids occur through their action on cell membranes, particularly gram-positive bacteria. (Maia et al. 2007).

The inhibitory response of lipids on methane production depends on concentration, type, fatty acid composition, and nutrient composition of diets (Beauchemin et al. 2008; Machmüller, 2006). Despite the possibility of a methane reduction greater than 40% when high levels of lipids are added (Machmuller and Kreuzer, 1999; Jordan et al, 2006b), a reduction from 10 to 25% is more likely to be obtained (Beauchemin et al. 2008).

Jordan et al. (2006b) reported that feeding cows with diets combined between whole soybean or refined soy oil decreased enteric methane by approximately 25% and 39%, respectively. Grainger et al. (2010) who included cottonseed in the diet and McGinn et al. (2004) who studied sun flower oil supplementation, observed reduced methane emissions by 17% and 21% respectively.

2.4. COTTONSEED

Supplementation of diets with lipids is one recognized mitigation strategies due its effectiveness in reducing CH_4 and animal productivity (Hristov et al. 2013).

Feeding whole seeds, a byproduct from the plant production, as well as pure oil, are tools to increase the dietary lipid concentration, but the difference in physical form might influence the effect of lipid in the rumen (Brask et al. 2013).

Oil in seeds is stored intracellularly, and the lipid release depends on the digestion and breakdown of the cell wall, which leads to a slower release compared with feeding oil directly (Steele et al. 1971). Rumen bypass fatty acids has no negative effects on ruminal fermentation and nutrient digestibility (Litherland et al. 2005; Kazama et al. 2010; Côrtes et al. 2011). Encapsulation of fatty acids within the oilseed has additional benefit of lessening detrimental effect of lipid on digestion, which is the major limiting factor in lipid utilization (Jenkins and Lundy, 2001).

Cottonseed is a by-product of the cotton industry. This feedstuff is of significant feeding value (Chandler, 1992). Cottonseed in dry matter content is high in fat (200 g/kg), crude protein (230 g/kg) and neutral detergent fiber (440 g/kg) (NRC, 1989).

The supplemental lipid from cottonseed increases the energy density of the diet and the fiber provided by the lint and the hull from cottonseed has been shown to be a good source of effective fiber (Clark et al. 1993). It is commonly used in diets for high producing dairy cows to increase the energy density and maintain acceptable fiber concentrations (Coppock et al. 1987). Thus, cottonseed can be used as a source of energy, protein and fiber, in addition has a potential option as a dietary supplement to reduce CH_4 emissions.

2.5. VITAMIN E

Vitamin E is lipid-soluble and it is used in animal feed mainly due to the antioxidant function (Baldi, 2005) and because it assists in reducing the effects of oxidative stress. Vitamin E is lipid-soluble and it is used in animal feed mainly due the antioxidant function (Baldi, 2005) and because it assists in reducing the effects of oxidative stress. Thus, can be important to supply additional antioxidants to the diet of cattle when oil and oilseeds will be included.

Many factors interact to determine variable effects of lipid supplementation on animal performance, on extent of ruminal biohydrogenation, and the nutritional and sensorial properties of dairy and meat products (Chilliard and Ferlay, 2004).

Supplemental vitamin E enhanced the antioxidation and palatability characteristics of beef (Bloomberg et al. 2011), elevated α-tocopherol concentration in

plasma and suppressed oxidation of lipid in muscle tissue of steers (O'Grady et al. 2001), improved the function of bovine neutrophils and reduced somatic cell counts in milk of dairy cows (Politis et al. 2004),

Lipid supplementation of cattle may also increase the risk of blood lipoperoxidation in a similar manner to that of milk and meat, and expose the animals to the deleterious effects of oxidative stress (Gobert et al. 2009)

The rumen environment is relatively free of oxygen and suitable for the colonization and growth of rumen microbes which are anaerobes. However, a small amount of oxygen, which is harmful to rumen microbes, may go into the rumen with saliva, feeds, drinking water and diffusion from blood into rumen. Therefore, supplementing vitamin E to relieve oxidation effects from oxygen could be beneficial to rumen microbes and consequently feed digestion (Wey et al. 2015).

Indeed, feeding 200 mg/kg of dry matter of a synthetic antioxidant (blend of ethoxyquinand tertiary-butyl-hydroquinone) to dairy cows has improved the utilization of diets containing both oxidized and fresh fat by increasing fiber and carbohydrate digestibility (Vázquez-Anón and Jenkins, 2007).

In *in vitro* rumen fermentation, Hou et al. (2013) reported that supplementing vitamin E at 2 mg/80 mL of incubation liquid increased *in vitro* rumen acetate and total SCFA production and decreased butyrate production. Naziroglu et al. (2002) observed that supplementing vitamin E at 0.8 mg/100 mL of incubation liquid increased acetate and propionate production. Hino et al. (1993) reported that adding β-carotene plus vitamin E (α-tocopherol) at 5 mg/mL, improved cellulose digestion at the presence of 100 mg/L of safflower oil.

However, no studies have assessed the effects of supplemental vitamin E in conjunction with high cottonseed levels in the cow's diet. It is unclear if supplementing vitamin E would be beneficial to rumen fermentation.

2.6. FECES METHANE EMISSIONS AND ANAEROBIC DIGESTION

Many factors as species, diet, storage temperature, type of storage and farming system can influence the production of CH_4 from feces. Among these diet is the crucial factor for GHG emissions from feces. Various researches has shown that composition of

the diet fed to animals like concentrate, forage proportions, fat content, crude protein content and other feed supplements influenced CH_4 emissions from feces (Nampoothiri et al. 2015).

So far, many investigations have quantified feeding effects on enteric methane emissions, but few have measured dietary effects on feces-derived methane, and even fewer have simultaneously quantified both enteric and feces-derived methane (Ku et al. 2002; Boadi et al. 2004; Hindrichsen et al. 2005; Mwenya et al. 2005).

On pasture, methane formation in cattle feces is very low (Jarvis et al. 1995) and it is often neglected when quantifying methane emission from feces (IPCC, 1996). However, when stored anaerobically, feces can produce from 7 to 27% of total methane emission in ruminants (Ku et al. 2002; Hindrichsen et al. 2006) Therefore, it is globally seen as an important source of methane emission.

One of the most common biological processes in nature is the production of biogas from organic matter in the absence of oxygen (Mata-Alvarez et al., 2000). Within an anaerobic digester, the process of converting organic matter into methane is broken down into 3 main steps. The first step is the acidogenesis that converts complex organic matter into intermediary products, primarily SCFA such as acetate, propionate, and butyrate. The organic matter is composed of a mix of lipids, proteins, and carbohydrates. Acidogenesis is completed by fermentative bacteria (Franco et al., 2007). After acidogenesis, acetogenic bacteria convert SCFA with more than 2 carbons to acetate, carbon dioxide and hydrogen gas and this step is called acetogenesis. Finally, methanogenic bacteria convert the acetate to methane. Methanogens are also able to convert hydrogen and carbon dioxide into methane. Approximately ¾ of the methane produced comes from the cleavage of acetic acid into methane and carbon dioxide (FAO, 1997).

First one, Hashimoto et al. (1981) reported that when diet changed from 92% to 7% forage there was an increase in CH_4 emissions per unit of volatile solids in bovine feces. Lodman et al. (1993) compared feces from feedlot steer receiving diets composed of forage only or a high grain diet (11% forage) and found seven times higher methane emissions per unit of organic matter in feces originating from the latter diet.

According to IPCC (2006), use of a lipid rich diet results in higher emissions of CH_4 during the storage of feces than other diets. Kulling et al. (2002) supplemented dairy cows in early lactating with lauric acid (40 g/kg of DM), authors observed anti enteric methanogenic activity and found that feces of cows supplemented with lauric

acid had higher CH_4 emissions as compared to those supplemented with stearic acid. Moller et al. (2012) also observed higher CH_4 values using high fat concentrate (rape seed) when compared to low fat concentrate (maize).

Nonetheless, most dietary strategies designed to mitigate enteric methane remains still unclear whether feces-derived methane facilitates mitigation, is neutral or even compensates for achievements made in the digestive tract of the ruminants (González-Avalos et al. 2001).

Anaerobic digestion has proved to be an effective technology to feces treatment due capacity in biodegradable organic matter with high moisture, carbohydrate, lipid and protein contents. In addition, anaerobic digestion can recover the feces energy content in the form of methane (Zhang et al. 2014).

The biochemical methane potential (BMP) tests are a widely accepted protocol for estimating methane yield from feces. Thus, studies about feces methane emissions from different diets are important information to support GHG inventory.

REFERENCES

ALCOCK, D. J.; HEGARTY, R. S. Potential effects of animal management and genetic improvement on enteric methane emissions, emissions intensity and productivity of sheep enterprises at Cowra, Australia. **Animal Feed Science and Technology**, v. 166-67, p. 749-760, 2011.

BALDI, A. Vitamin E in dairy cows. **Livestock Production Science,** v. 98, p. 117-122, 2005.

BAUMAN, D. E.; CAPPER, J. L. Future challenges and opportunities in animal nutrition. In: SOUTHWEST NUTRITION & MANAGEMENT CONFERENCE, 26th, 2011, Tempe, AZ. **Proceedings…** Tempe: University of Arizona, 2011. p. 70-84.

BEAUCHEMIN, K. A.; KREUZER, M.; O'MARA, F.; MCALLISTER, T. A. Nutritional management for enteric methane abatement: a review. **Australian Journal of Experimental Agriculture**, v. 48, n. 1/2, p.21-27, 2008.

BEAUCHEMIN, K. A.; JANZEN, H. H.; LITTLE, S. M.; MCALLISTER, T. A.; MCGIM, S. M. Mitigation of greenhouse gas emissions from beef production in western Canada: evaluation using farm-based life cycle assessment. **Animal Feed Science and Technology,** v. 166/167, p. 663-77, 2011.

BLAXTER K. L.; CZERKAWSKI, J. Modifications of the methane production of the sheep by supplementation of its diet. **Journal of the Science of Food and Agriculture**, v. 17, p.417-421, 1966.

BLOMMBERG, B. D.; HILTON, G. G.; HANGER, K. G.; RICHARDS,C. J.; MORGAN, J. B.; VANOVERBEKE, D. L. Effects of vitamin E on color stability and palatability of strip loin steaks from cattle fed distillers grains. **Journal of Animal Science**, v. 89, p. 3769-3782, 2011.

BOADI, D.; BENCHAAR, C.; CHIQUETTE, J.; MASSÉ, D. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. **Canadian Journal of Animal Science**, v. 84, p. 319-335, 2004.

BRASK, M.; LUND, P.; WEISBJERG, M. R.; HELLWING, A. L. F.; POULSEN, M.; LARSEN, M. K.; HVELPLUND, T. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. **Journal of Dairy Science,** v. 96, n. 4, p. 2356-2365, 2013.

BURKEN, D. B.; HICKS, R. B.; VANOVERBEKE, D. L.; HILTON, G. G.; WAHRMUND, J. L.; HOLLAND, B. P.; KREHBIEL, C. R.; CAMFIELD, P. K.; RICHARDS, C. J. Vitamin E supplementation in beef finishing diets containing 35% wet distillers grains with solubles: feedlot performance and carcass characteristics. **Journal of Animal Science**, v. 90, p. 1349-1355, 2012

CHILLIARD, Y.; FERLAY, A. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. **Reproduction Nutrition Development,** v.44, p.467-492, 2004.

CHUNTRAKORT, P.; OTSUKA, M.; HAYASHI, K.; SOMMART, K. Effects of oil plant use for rumen methane mitigation in in vitro gas production. **Khon Kaen Agricultural Journal**, v. 39, p. 246-250, 2011.

CIESLAK, A.; MILTKO, R.; BELZECKI, G.; SZUMACHER-STRABEL, M.; POTANKI, A.; KWIATOWSKA, E.; MICHALOWSKI, T. Effect of vegetable oils on the methane concentration and population density of the rumen ciliate, Eremoplastron dilobum, grown in vitro. **Journal of Animal Feed Science**, v. 15, p. 15-18, 2006.

CLARK, P. W.; ARMENTANO, L. E. Effectiveness of neutral detergent fiber in whole cottonseed and dried distillers grains compared with alfalfa haylage. **Journal of Dairy Science**, v. 76, p. 2644-2650, 1993.

COPPOCK, C. E.; LANHAM, J. K.; Horner, J. I. A review of the nutritive value and utilization of whole cottonseed, cottonseed meal and associated by-products by dairy cattle. **Animal Feed Science and Technology**, v. 18, n. 2, p. 89-129, 1987.

CRUICKSHANK, G. J.; THOMSON, B. C.; MUIR, P. D. Modelling management change on production efficiency and methane output within a sheep flock. **Sustainable land management and climate change**. Wellington, NZ: Ministry of Agriculture and Forestry, 2008.

DOREAU, M.; FERLAY, A. Effect of dietary lipids on nitrogen metabolism in the rumen: a review. **Livestock Production Science**, v. 43, p. 97-110, 1995.

EPA (ENVIRONMENTAL PROTECTION AGENCY). **Global anthropogenic non-CO_2 greenhouse gas emissions: 1990-2020.** Washington: EPA, 2006.

FAO (FOOD and AGRICULTURE ORGANIZATION). **Greenhouse gas emissions from pig and chicken supply chains:** a global life cycle assessment. Rome: FAO, 2013.

FAO (FOOD AND AGRICULTURE ORGANIZATION). **Greenhouse gas emissions from ruminant supply chains: a global life cycle assessment**. Rome: FAO, 2013.

FAO (FOOD AND AGRICULTURE ORGANIZATION). **Livestock's long shadow: environmental issues and options**. Rome: FAO, 2006.

FAO (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS). Methane production 1997. In: MIYAMOTO, K., Ed. **Renewable biological systems for alternative sustainable energy production.** Chapter 4. Available from: <www.fao.org/docrep/w7241e/w7241e00.htm>. Accessed: Sept. 13, 2016.

FAO (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS) **The state of food and agriculture: livestock in the balance**. Rome: FAO, 2009.

FERGUNSON, M.; THOMPSON, A.; GORDON, D. Ewe management handbook: optimising Merino ewe nutrition to increase farm profit for the high rainfall zone (Ed). Victoria: Lifetimewool, 2007.

FRANCO, A.; MOSQUERA-CORRAL, A.; CAMPOS, J. L.; ROCA, E. Learning to operate anaerobic bioreactors. In: MÉNDEZ-VILAS, A. (Ed.). **Communicating current research and educational topics and trends in applied microbiology.** Badajoz: Formatex, 2007**.** p. 618-627.

GERBER, P. J.; STEINFELD, H.; HENDERSON, B.; MOTEET, A.; OPIO, C.; DIJKMAN, J.; FALCUCCI, A.; TEMPIO, G. **Tackling climate change through livestock**: a global assessment of emissions and mitigation opportunities. Rome: FAO, 2013.

GOBERT, M.; MARTIN, B.; FERLAY, A.; CHILLIARD, Y.; GRAULET, B.; PRADEL, P.; BAUCHART, D.; DURAND, D. Plant polyphenols associated with vitamin E can reduce plasma lipoperoxidation in dairy cows given n-3 polyunsaturated fatty acids. **Journal of Dairy Science**, v. 92, p. 6095-6104, 2009.

GONZALES-AVALOS, E.; RUIZ-SUAREZ, L. G. Methane emission factors from cattle manure in Mexico. **Bioresource Technology**, v. 80, p. 63-71, 2001.

GRAINGER, C.; WILLIAMS, R.; CLARKE, T.; WRIGHT, A. D. G.; ECKARD, R. J. Supplementation with whole cottonseed causes long-term reduction of methane

emissions from lactating dairy cows offered a forage and cereal grain diet**. Journal of Dairy Science**, v. 93, n. 6, p. 2612-2619, 2010.

HASHIMOTO, A.G.; CHEN, Y.R.; VAREL, V.H. Theoretical aspects of CH_4 production: state of the art. In: INTERNATIONAL SYMPOSIUM ON LIVESTOCK WASTES, A RENEWABLE SOURCE, 4th, Amarillo, 1980. **Proceedings…** St. Joseph: ASAE, 1981. p. 86-89, 1981.

HERRERO, M. Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. **Proceedings of the National Academy of Sciences**, v. 110, p. 20888-20893, 2013.

HINDRICHSEN, I.K. Effect of the carbohydrate composition of feed concentrates on methane emission from and their slurry. **Environmental Monitoring and Assessment,** v. 107, p. 329-350, 2005.

HINDRINCHSEN, I. K.; WETTSTEIN, H. R.; MACHMULLER, A.; KREUSER, M. Methane emission, nutrient degradation and nitrogen turnover in dairy cows and their slurry at different milk production scenarios with and without concentrate supplementation. **Agriculture, Ecosystems & Environment**, v. 113, n. 1/4, p. 150-161, 2006.

HINO, T.; ANDOH, N.; OHGI, H. Effects of β-carotene and α-tocopherol on rumen bacteria in the utilization of long-chain fatty acids and cellulose. **Journal of Dairy Science**, v. 76, n. 2, p. 600-605, 1993.

HOU, J .C.; WANG, F.; WANG, Y .T.; LIU, F. Effects of vitamin E on the concentration of conjugated linoleic acids and accumulation of intermediates of ruminal biohydrogenation in vitro. **Small Ruminant Research**, v. 111, p. 63-70, 2013.

HRISTOV, A. N. Mitigation of methane and nitrous oxide emissions from animal operations: III a review of animal management mitigation options. **Journal of Animal Science**, v. 91, p. 5095-5113, 2013.

IPCC (INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE). **Revised 1996 IPCC guidelines for national greenhouse gas inventories.** Geneva: IPCC, 1996. v. 3, Reference manual.

IPCC (INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE)**Working group I contribution to the IPCC fifth assessment report climate change 2013:** the physical science basis: final draft underlying scientific-technical assessment. Available

from: <https://www.ipcc.ch/pdf/assessment-report/ar5/wg1/drafts/fgd/WGIAR5_WGI-12Doc2b_FinalDraft_TechnicalSummary.pdf>. Acessed: 28 July 2016.

JARVIS, S. C.; LOVELL, R. D.; PANAYIDLES, R. Patterns of methane emission from excreta of grazing animals. **Soil Biology and Biochemistry**, v. 27, p. 1581-1588, 1995.

JOHNSON, K. A.; JOHNSON, D. E. Methane emissions from cattle. **Journal of Animal Science**, v. 73, p. 2483-2492, 1995.
Livestock's Long Shadow: Environmental Issues and Options (FAO, 2006).

JORDAN, E.; KENNY, D.; HAWKINS, M.; MALONE, R.; LOVETT, D. K.; O'MARA, F. P. Effect of refined soy oil or whole soybeans on intake, methane output, and performance of young bulls. **Journal of Animal Science**, v. 84, p. 2418-2425, 2006.

KAMRA, D. N. Rumen microbial ecosystem. **Current Science,** v. 89, p. 124-135, 2005.

KNAPP, J. R. Enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. **Journal of Dairy Science**, v. 97, p. 3231-3261, 2014.

KRAUSE, D. O. Rumen microbiology: leading the way in microbial ecology. **Journal of Animal Science**, v. 91, p. 331-341, 2013.

KULLING, D. R. Methane emissions from differently fed dairy cows and corresponding methane and nitrogen emissions from their manure during storage. **Environmental Monitoring and Assessment,** v. 79, n. 2, p. 129-150, 2002.

KURIHARA, M.; MAGNER, T.; HUNTER, R. A.; MCGRABB, G. J. Methane production and energy partition of cattle in the tropics. **British Journal of Nutrition**, v. 8, n. 3, p. 227- 234, 1999.

LODMAN, D. W. Estimates of methane emission from manure of U.S. cattle. **Chemosphere,** v. 26, p.189-199, 1993

LOVETT, D.; LOVELL, S.; STACK, J.; CALLAN, J.; FINLAY, M. ; CONOLLY, J.; O'MARA, F. P. Effect of forage/concentrate ratio and dietary coconut oil level on methane output and performance of finishing beef heifers. **Livestock Production Science**, v. 84, p. 135-146, 2003

MACHMULLER, A.; OSSOWSKI, D. A.; KREUZER M. Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. **Animal Feed Science and Technology**, v. 85, n.1/2, p.41-60, 2000.

MACHUMULLER, A. Medium-chain fatty acids and their potential to reduce methanogenesis in domestic ruminants. **Agriculture, Ecosystems & Environment,** v. 112, n. 2/3, p. 107-114, 2006.

MACHUMULLER, A.; SOLIVA, C. R.; KREUSER, M. Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. **British Journal of Nutrition,** v. 90, n. 3, p. 529-540, 2003.

MAIA, M. R. G.; CHAUDHARY, L. C.; FIGURES, L.; WALLACE, R. J. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. **Antonie Van Leeuwenhoek,** v. 91, n. 4, p. 303-314, 2007.

MARTIN, C.; ROUEL, J.; JOUANY, J. P.; DOREAU, M.; CHILLIARD, Y. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. **Journal of Animal Science**, v. 86, n. 10, p. 2642-2650, 2008.

MATA-ALVAREZ, J.; MACE, S.; LLABRES. P. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. **Bioresource Technology,** v.74, p. 3-16, 2000.

MCALLISTER, T. A.; NEWBOLD, C. J. Redirecting rumen fermentation to reduce methanogenesis. **Australian Journal of Experimental Agriculture,** v.48, p. 7-13, 2008.

MCGINN, S. M.; BEAUCHEMIN, K. A.; COATES, T.; COLOMBATTO, D. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. **Journal of Animal Science**, v.82, n. 11, p. 3346-3356, 2004.

MITSUMORI, M. Responses in digestion, rumen fermentation and microbial populations to inhibition of methane formation by a halogenated methane analogue **British Journal of Nutrition,** v.108, n. 3, p. 482-491, 2012.

MØLLER, H.B.; SARKER, S.; HELLWIN, A.L.F.; WEISBJERG, M.R. Quantification of methane production and emission from anaerobic digestion of cattle manure derived

from different feeding. In: CIGR INTERNATIONAL CONFERENCE OF AGRICULTURAL ENGINEERING, 3, **,** Valencia, 2012. **Proceedings**… Kyoto: CIGR, 2012. p. 8-12.

MWENYA, B.; SAR, C.; PEN, B.; MORIKAWA, R.; TAKAURA, K.; KOGAWA, S.;Takahashi, J. Effect of feed additives on ruminal methanogenesis and anaerobic fermentation of manure in cows and steers. In: **International Congress Series**, v. 27, p. 209-212, 2006

NAGAJARA, T. G.; NEWBOLD, C. J.; VAN NEVEL, C. J.; DEMEYER, D. I. Manipulation of ruminal fermentation. In: HOBSON, P. N; STEWART, C. S. (Ed.). **The rumen microbial ecosystem**. London: Blackie Academic & Professional; 1997. p. 523-632.

NAMPOOTHIRI, V. M.; MOHINI, M.; THAKUR, S. S.; MONDAL, G. influence of diet on methane and nitrous oxide emissions from cattle manure. **Asian Journal of Atmospheric Environment**, v.9, n. 3, p. 187-193, 2015

NAZIROGLUI, M.; GULER, T.; YUCE, A. Effect of vitamin E on ruminal fermentation in vitro**. Journal of Veterinary Medicine,** v. 49, p. 251-255, 2002.

O'GRADY, M.N.; MONAHAN, F.J.; FALLON, R.J.; ALLEN, P. Effects of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. **Journal of Animal Science**, v. 79, p. 2827-2834, 2011.

POLITILIS, I.; BIZELIS, I.; TSIARAS, A.; BALDI, A. Effect of vitamin E supplementation on neutrophil function, milk composition and plasmin activity in dairy cows in a commercial herd. **Journal of Dairy Research,** v. 71, p. 273-278, 2004.

THORNTON, P.K. Livestock production: recent trends, future prospects. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 365, n. 1554, p. 2853-2867, 2010.

TUBIELLO, F. N. The FAOSTAT database of greenhouse gas emissions from agriculture. **Environmental Research Letters,** v.8, p. 15-19, 2013.

SMITH, P. How much land based greenhouse gas mitigation can be achieved without compromising food security and environmental goals? **Global Change Biology,** v. 19, p. 2285-2302, 2013.

VASQUES-ANON, M.; JENKINS, T. Effects of feeding oxidized fat with or without dietary antioxidants on nutrient digestibility, microbial nitrogen, and fatty acid metabolism. **Journal of Dairy Science,** v. 90, p. 4361-4367, 2007.

WEI, C.; LIN, S. X.; WU, J. L.; ZHAO, G. Y.; ZHANG, T. T.; ZHENG, W. S. Effects of supplementing vitamin E on in vitro rumen gas production, volatile fatty acid production, dry matter disappearance rate, and utilizable crude protein. **Czech Journal of Animal Science**, v. 60, n. 8, p. 335-341, 2015.

YOUNG, J. M.; THOMPSON, A. N.; CURNOW, M.; Oldham, C.M. Whole-farm profit and the optimum maternal liveweight profile of Merino ewe flocks lambing in winter and spring are influenced by the effects of ewe nutrition on the progeny's survival and lifetime wool production. **Animal Production Science,** v. 51, p. 821-833, 2011.

3. NUTRIENTS DIGESTIBILITY AND CHANGES IN THE INGESTIVE FEEDING BEHAVIOR OF COWS FED COTTONSEED AND VITAMIN E

Abstract: High lipid concentration on ruminant diets often impairs nutrients digestibility and feed intake. A protected lipid source and an antioxidant additive can be an alternative to improve diet energy without disadvantage for the animal production. The objective of this study was to evaluate the dry matter intake (DMI), nutrient digestibility and feeding behavior of cows fed cottonseed and vitamin E. Six cannulated, non-pregnant, nonlactating cattle were distributed in a replicated 3 x 3 Latin Square design. Treatments were: 1) Control, 2) CS: 30% cottonseed included; 3) CSVitE: 30% cottonseed plus 500 IU VitE included. Data were analyzed by SAS (v9.3) and the significance was declared at $P \leq 0,05$. Results were compared through orthogonal contrasts, where contrast 1: control vs. CS and VitE, and contrast 2: CS vs. CSVitE. Diets with cottonseed had 17% greater digestibility of ether extract and 9% lower digestibility of non-fibrous-carbohydrates compared to the control. Cows from treatments with cottonseed spent 13% higher time eating, 48% more ruminating, 34% more chewing and 17% lower time in idles compared to the control. Ruminal solid mass was 26% higher and ruminal total mass was 8% higher for diets with cottonseed compared to the control. Including cottonseed in a diet at 30% had positive effects on feeding behavior it was enough to avoid reductions in the DMI or nutrient digestibility. The inclusion of vitamin E had no effect on ruminal fermentation, nutrient digestibility and feeding behavior, so it is not recommended their use due the absence of favorable results.

Keywords: Antioxidant, lipid, pH, ruminal kinetics

3.1. INTRODUCTION

Feeding behavior is a highly relevant tool to evaluate diets because it provides feed management of animals for better production (Cavalcanti et al. 2008). Daily activities are characterized by three basic types of behavior: feeding, rumination and

idleness, and their duration and distribution may be affected by diet, management and climatic conditions (Fisher et al. 1997). Feed intake is a function of both meal size and meal interval, it is determined by satiety and hunger and can influence feed digestion and rate through the gastro-intestine tract (Allen, 2000).

Adding lipids to cattle affects the nutrients ruminal digestion and ruminal disappearance rate, which could affect ruminal digestive pool size and rumination activity (Harvatine and Allen, 2005). In addition, a decrease in the feed intake due to the liberation of fatty acids that act as a physiological signal to decrease meal size or increase meal interval (Harvatine and Allen, 2006) can be observed when lipid are included.

Oil in seeds is stored intracellularly, and the lipid release depends on the digestion and breakdown of the cell wall, which leads to a slower release compared with feeding oil directly (Steele et al. 1971). A protected lipid has less negative effects on ruminal fermentation and nutrient digestibility (Litherland et al. 2005; Kazama et al. 2010; Côrtes et al. 2011).

Cottonseed is a source of protected lipid that gather high concentration of oil, protein and fiber (Pesce, 2008), these nutrients are important for the ruminants and it explain their utilization for the cows. However, the inclusion of lipid can have use limited by possibility of unsaturated fatty acids in suffer lipid peroxidation (Zakrys et al. 2008). Dietary lipids such as supplemental oil or oilseeds, if not stabilized, can be significant contributors to the load of free radicals in the animal (Andrews et al. 2006). In addition, a small amount of oxygen may go to the rumen with saliva, feeds, drinking water and diffusion from blood into rumen, it has as consequences a release free radicals in the rumen (Wey et al. 2015).

A main way to reduce or prevent lipid peroxidation is through the use of antioxidants. In recent years, supplemental vitamin E in the diet of ruminants has been studied for its potential role in (Bloomberg et al. 2011).

Generation of free radicals can damage cells and can impair in the animal production (Miller and Brezeinska-Slebodizinska, 1993). Therefore, supplementing vitamin E to relieve oxidation effects from free radicals could be beneficial to rumen microbes and consequently improve rumen fermentation, nutrient digestibility and it could change feeding behavior (Wey et al. 2015).

To ascertain this, an experiment was designed to study the effects of cottonseed and vitamin E inclusion in the diets of cow on nutrient intake, digestibility and excretion, ruminal dynamics and feeding behavior.

3.2. MATERIAL AND METHODS

3.2.1. Study location and ethical issue

The study was conducted at the University of Sao Paulo, Pirassununga, Brazil. The experiment was approved by and complied with the guidelines set out by the Ethics Committee in the Use of Animals of the University of São Paulo, under application number nº 009/2013, in respect to animal experimentation and care of animals used for scientific purposes.

3.2.2. Animal, housing and feeding

Six Holstein cows, not pregnant and non-lactating, with rumen fistula and mean body weight of 876 kg (±16.1) were arranged in individual pen with free access to water and sand bedded. Animals were fed ad libitum twice daily (08h00 and 16h00). Feed was weighed daily and offered to each animal after orts from the previous day had been removed. The vitamin E amount was weighted daily to offer 500 IU per animal per day. Orts were recorded once daily, before feeding, and the feeding rate was adjusted to yield orts on the basis of at least 5% of the amount supplied (on an as-fed basis). The animals were weighed individually on the initial and final day of each experimental period.

3.2.3. Experimental design and treatments

Animals were arranged in a replicated 3 x 3 Latin Squared design with 3 periods; each experimental period lasted 21 days. Three dietary treatments were as follows: 1) Control: diet without treatment; 2) CS: diet added with 30% of cottonseed and 3) CSVitE: diet added with 30% of cottonseed plus 500IU vitamin E. The vitamin E level was established according to Baldwin et al. (2010) and Montgomery et al. (2005). The vitamin E source was Lutavit E 50 BASF, with 50% alpha tocopheryl acetate. Vitamin E was weighed daily according to the amount of dry matter that would be offered. This was mixed and homogenized in the concentrate and offered to the animals.

The ingredient and chemical composition of the experimental diets are given in Table 1.

Table 1.Ingredients and chemical composition of dietary treatments

	Dietary treatments		
	Control	CS	CSVitE
Ingredient			
Suggarcane bagasse, (g/kg DM)	134	134	134
Cottonseed, (g/kg DM)	-	304	304
Ground corn grain, (g/kg DM)	572	281	281
Citrous polp, (g/kg DM)	183	183	183
Soybean meal, (g/kg DM)	81.7	81.7	81.7
Minerals, (g/kg DM)	60	60	60
Limestone, (g/kg DM)	40	40	40
Urea, (g/kg DM)	137	27	27
Vitamin E,(IU)	-	-	500
Chemical composition			
DM, (g/kg)	891	910	910
CP, (g/kg DM)	158	160	160
EE, (g/kg DM)	26,1	76,9	76,9
NDF, (g/kg DM)	234	357	357
ADF,(g/kg DM)	171	265	265
Lignin, (g/kg DM)	55,3	136	136
Ca, (g/kg DM)	15,7	18,2	18,2
P, (g/kg DM)	12,7	14,7	14,7
Hemicellulose[1], (g/kg DM)	63,0	92,0	92,0
Cellulose[2], (g/kg DM)	115	136	136
OM[3], (g/kg DM)	829	845	845

NFC[4], (g/kg DM)	525	328	328
Gross energy, (Mcal/kg of DM)	4,15	4,27	4,27
Vitamin E, (UI/kg)	514	507	507

DM: dry matter; OM: organic matte;. CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: Non-fibrous carbohydrate; GE: gross energy; Mcal: mega calorie.
[1] Hemicellulose: NDF − ADF.
[2] Cellulose: ADF − Lignin.
[3] OM: DM-mineral
[4] NFC: 100 − (CP + NDF + crude fat + ash).
Font: Nogueira, 2017.

3.2.4. Sampling schedule

The trial consisted of three experimental periods, each lasting 21 days. The first 10 days of each period were used for adaptation. The 15th day was used for feeding behavior, and the 16th was used for pH evaluation. The 11th to 15th days were used to obtain DMI. On the 11th to 17th days nutrient digestibility, using external marker and feces collection, was done. The 20th to 21th days were used for ruminal dynamic information.

3.2.5. Feed intake

Feed intake was determined between days 11 on 15 of each period by weighing feeds offered to and refused by the animals. During the feed intake determination, feed ingredients samples were collected and stored at −20 °C. Individual feed ingredients were composited in representative samples on an equal-weight basis. Pool samples was dried at 60°C (forced-air oven) for 48 h and ground to pass a 1-mm Wiley mill screen and analyzed for DM, CP, EE, NDF, ADF, lignin and gross energy.

3.2.6. Ruminal dynamics

The last 2 days of each period, before morning feeding and 3 hours after morning feeding, ruminal content was manually removed according to Chilibroste et al. (2000). Ruminal content was separated, manually through a screen, in solid and liquid content. After that, these were weighed to determine total ruminal solid and liquid contents. Then, ruminal solid and liquid content were sampled. Immediately after this, ruminal content was replaced in the rumen. Solid and liquid samples were dried at 60°C (forced-air oven) for 72 hours to determine the dry matter of each compound. The solid and liquid volume was adjusted by dry matter. Starting ruminal solid and liquid mass was calculate according Robinson et al (1987):

$$SD, g/kg.h = 100 \times \frac{\text{DMI (kg.day)}}{\text{Solid Mass (kg)}} \div 24$$

$$SD, kg/h = \text{Solid mass (kg)} \times \frac{SD\ (\%/hour)}{100}$$

Where:

SD: solid disappearance rate.

3.2.7. Feeding behavior

Eating, ruminating and idleness activities, measured in minutes, were monitored visually over a 24-h period. Animals were considered to be at eating activity (min/day) when they had the head in the feed bunk and were in contact with the diet. Rumination activity (min/day) included regurgitation, re-mastication, and re-swallowing. Idles activity (min/day) included periods during which the animals slept, lay down, walked or stood idly.

Activities were noted every 5 min, and each behavior was assumed when this to persisted for the entire 5-min interval. Total chewing time was calculated as the sum of eating and ruminating time (Maekawa et al. 2002).

A meal or bout was defined when a minimum sequence of two activities of the same behavior persisted. The total time spent eating, ruminating or idles, as min/day, was calculated by sum of activities over the day. Length, as min/meal or min/bout, was calculated by division between total time spent on each behavior (min) and how many meals or bouts the animals had over the day.

DM or NDF content (%) was used to calculate the amount of DM and NDF eaten, ruminated or chewed. DM or NDF, when express as DM/min or NDF/min, were determined using total DM or NDF intake, ruminated or chewed (kg) divided by total time eating, ruminating or chewing (min). DM or NDF, when express as DM/bout or NDF/bout, were determined using total DM or NDF intake, ruminated or chewed (kg) divided by the number of eating, ruminating or chewing bouts per day, as described by Bürger et al. (2000).

3.2.8. Nutrients digestibility

Total apparent digestibility of nutrients was determined using chromium oxide. During days 7th to 11th external market was inserted into the rumen, via rumen fistula, to ensure a stable market concentration. During days 12th until 17th, 15 g/head/d indigestible marker was placed twice daily (08h00 and 16h00 before feeding) via rumen fistula. Feces were collected, via rectal, twice daily, during days 12th until 17th at 08h00 and 16h00 after feeding. A composite of 200g samples were then analyzed for chromium oxide concentration according to Conceição et al. (2007). Nutrient digestibility (%) was calculated as:

$$Digestibility = 100 - (\frac{chromiun\ in\ the\ diet \div nutrient\ intake \times 100}{chromiun\ in\ the\ feces}) \times 100$$

Fecal output and nutrient excretion, express as kg (DM basis), was calculated as equation:

$$Fecal\ output = \frac{(100\ - nutrient\ digestibility) \times nutrient\ intake}{100}$$

3.2.9. pH evaluation

Ruminal pH was obtained using a data logger model T7-1 LRCpH, Dascor, Escondido, CA. Attached on data logger, 2 weighs of 900 grams each, were used to maintain the device in the position in the rumen ventral sacral. pH meters unit were calibrated to 7.0 and 4.0 pH before being put into the rumen. At 16th day, during 24 hours, pH was measured each 10 minutes. Data were uploaded in a computer and the Excel program was used to arrange data.

3.2.10. Laboratory analyses

Individual feed ingredients, orts and feces were collected in each period and composited in representative samples. Samples were dried at 60°C during 48 hours and milled through a one-mm screen using a Willey mill. The DM content was determined at 100°C for 4 h followed by cold weighing (method 930.15, AOAC, 1995). Nitrogen content was determined by the micro Kjeldahl method (AOAC, 1995) and then, it was multiplied by 6.25 to determine CP. EE was determined using light petroleum ether in the Soxhlet apparatus (method 920.39, AOAC, 1995). GE was determined by combustion using an adiabatic calorimeter bomb according to AOAC (1995). NDF, ADF and lignin were determined using the sequential method with Ankom® Filter Bag technique and heat stable α−amylase (method 973.18, AOAC, 1995).

3.2.11. Statistical analyses

The data were analyzed using the MIXED procedure of SAS (Statistical Analysis System, version 9.0) with animals inside period as the experimental unit. Data are presented as least squares means of standard error. The model included the fixed effect of treatment and random effect square, period, and animals within the square. These variables were analyzed using the following model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + A_l(S_k) + e_{ijk},$$

Where:

Y_{ijkl} = the dependent response variable

μ = the overall mean

T_i = treatment effect

P_j = period effect

S_k = square effect

$A_l(S_k)$ = animals within square effect

e_{ij} = the residual error term.

Non-significant (NS) was considered when P value was higher than 10%.

Contrast statements were used to evaluate differences between means of Control vs CS plus CSVitE (C1) as well as between CS vs CSVitE (C2) treatments. Statistical significance was declared at $P \leq 0,05$.

3.3. RESULTS

3.3.1. Nutrient intake, excretion and digestibility

DM intake, expressed as kilograms per day, percentage of BW or g/kg metabolic weight (MW), were similar among treatments. Adding cottonseed to diets,

regardless of vitamin E inclusion, increased dietary intake EE (1.0 vs 0.32 gk) NDF (4.6 vs.2.76 kg) and ADF (3.4 vs. 1.9 kg) and decreased dietary intake by NFC (4.4 vs. 7.9 kg) when compared to the control. OM, CP and GE intake were not affected by cottonseed or vitamin E inclusion (Table 2).

In relation to nutrients excretion, dry matter, crude protein, ether extract, organic matter, non-fiber-carbohydrates and gross energy had similar excretion. Animals fed with cottonseed excreted higher NDF (1.9 vs. 1.4 kg) and ADF (1.6 vs.1.1 kg), when compared to the control (Table 2).

Cottonseed inclusion improved the digestibility of EE (938 vs. 801 g/kg) and decreased digestibility of NFC (782 vs. 857 g/kg) when compared to the control. No effects were observed to digestibility of DM, CP, NDF, ADF, OM, TDN or GE (Table 2).

Table 2 - Nutrient intake and excretion, as well as apparent digestibility of cows fed dietary treatments

	Treatments				Probability	
	Control	CS	CSVitE	SEM	C1	C2
Daily feed intake						
DMI, (kg)	14,6	15,4	15,4	0,61	0,0649	NS
DMI, (% BW)	1,66	1,74	1,74	0,05	0,0710	NS
DMI, (g/kg BW)	90,6	95,1	95,2	2,85	0,0710	NS
CP, (kg)	1,78	2,11	2,12	0,11	0,0750	NS
EE, (kg)	0,32	1,01	0,99	0,08	0,0001	NS
NDF, (kg)	2,76	4,70	4,63	0,31	0,0002	NS
ADF, (kg)	1,97	3,44	3,39	0,23	0,0001	NS
NFC, (kg)	7,98	4,46	4,51	0,49	0,0006	NS
OM, (kg)	11,5	12,2	12,2	0,64	NS	NS
GE, (MJ)	2,11	2,45	2,45	0,28	NS	NS
Daily nutrient excretion						
DM, (kg)	3,67	4,02	4,06	0,19	NS	NS
CP, (kg)	0,48	0,56	0,54	0,03	NS	NS
EE, (kg)	0,06	0,05	0,06	0,003	NS	NS
NDF, (kg)	1,46	1,99	1,89	0,12	0,0395	NS
ADF, (kg)	1,11	1,68	1,64	0,11	0,0047	NS
NFC, (kg)	0,92	0,95	0,92	0,09	NS	NS
OM, (kg)	3,23	3,57	3,42	0,20	NS	NS
GE, (MJ)	0,27	0,28	0,28	0,009	NS	NS
Nutrient digestibility						
DM, (g/kg)	676	679	677	22,4	NS	NS
CP, (g/kg)	707	727	732	19,3	NS	NS

EE, (g/kg)	801	942	935	19,0	0,0003	NS
NDF, (g/kg)	478	558	575	36,7	0,0693	NS
ADF, (g/kg)	421	487	493	44,4	0,0797	NS
NFC, (g/kg)	857	781	783	17,1	0,0334	NS
OM, (g/kg)	694	699	708	21,9	NS	NS
GE, (g/kg)	668	680	689	23,3	NS	NS
NDT[2], (g/kg)	729	744	750	20,4	NS	NS

DMI: dry matter intake; BW: body weight; CP: crude protein: EE ether extract, NDF: neutral detergent fiber,NFC: no fiber carbohydrates; OM: organic matter; TDN: total digestible nutrients, GE: gross energy SEM: standard error of mean, BW: body weight, SEM= standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
$BW^{0.75}$: metabolic of the body weight;
[2]NDT: %CP(dig) + %CNF(dig) + %NDF(dig) + EE(dig)*2.25
Font: Nogueira, 2017

3.3.2. Feeding behavior

Data referent to activities number, total time per activity over the day and mean time per activity are presented in Table 3. Eating activities number (meal/day) and time spent on each meal (min/meal) were similar among the treatments. On average, animals had 6.3 visits to the feedbunk per day and spent 34.1 minutes on each meal. However, cottonseed treatments spent higher time eating over the day when compared to the control (217 vs. 190.8 min).

Animals fed cottonseed had greater ruminating number bouts per day (16.1 vs. 14.1), spent more time in each rumination (26.8 vs. 20.6 min) and spent more time in rumination over the day (433.7 vs. 291.6 min) when compared the control diet. In addition animals fed cottonseed had a greater number of chewing bouts per day (22.9 vs. 20.1), spent more time in each chewing (28.6 vs. 24.1 min) and spent more time over the day chewing (650 vs. 482 min) when compared to the control diet (Table 3)

Idles number bouts per day were not affected by cottonseed or vitamin E. On mean, animals had 22 idles bouts per day. However, the animals fed with cottonseed spent less time in each idles (45.4 vs. 49.5 min) and spent less time in idles over the day (793 vs. 961 min) when compared to the control diet (Table 3)

Animals fed CSVitE diet had higher number bouts of ruminating per day (17.1 vs. 15.1) and spent smaller time in each idles bout (24.5 vs. 29.1 min) when compared to the CS diet (Table 3).

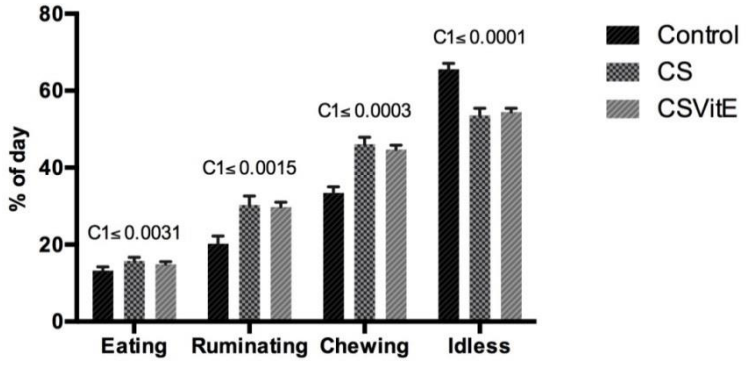
Table 3 - Meal, rumination, idles and chewing patterns of cows influenced by cottonseed and vitamin E.

	Treatments				Probability	
	Control	CS	CSVitE	SEM	C1	C2
Eating						
Meal/day	6,00	6,83	6,16	0,31	NS	NS
Min/day	190,8	219,1	215,0	7,49	0,0031	NS
Length, (min/meal)	32,4	33,1	36,9	1,79	NS	NS
Ruminating						
Bouts/day	14,1	15,1	17,1	0,63	0,0056	0,0452
Min/day	291,6	437,5	430,0	22,1	0,0015	NS
Bouts length, (min/bout)	20,6	29,1	24,5	1,27	0,0056	0,0452
Idles						
Bouts/day	21,8	22,3	22,5	0,45	NS	NS
Min/day	961,6	788,3	799,9	23,0	0,0002	NS
Bouts length, (min/bout)	49,5	45,9	45,0	1,80	0,0033	NS
Chewing						
Bouts/day	20,1	22,0	23,8	0,66	0,0095	0,0858
Min/day	482,5	656,6	645,0	22,8	0,0003	NS
Bouts length, (min/bout)	24,1	30,0	27,3	0,96	0,0101	NS

SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
Font: Nogueira, 2017.

According to figure 1, animals fed with cottonseed spent over the day more time eating (15% vs. 13%) and ruminating (30% vs. 20%) and less time in idles (55% vs. 67%) when compared to the control (Fig. 1).

Figure 1 – Time spent activities in percentage of the day for animals fed different diets. Bars means standard error



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Eating, when expressed as DM.kg/min and DM.kg/bout, were not affected by cottonseed or vitamin E. Cottonseed treatments had a greater amount of NDF, when expressed and NDF.kg/min, (0.022 vs. 0.015 kg) when compared to the control. However, NDF when expressed as FDN/bout, it was similar among treatments (Table 4).

Ruminating activity, when expressed as DM.kg/min, was lower for animals fed cottonseed when compared to the control diet (0.036 vs. 0.050 kg). However, when expressed as DM/bout, no difference was observed among treatments. NDF ruminated, when expressed as NDF/bout, was higher for the cottonseed treatments when compared to the control (0.297 vs. 0.224 kg). No difference among treatments were observed for the NDF, when expressed as NDF.kg/min.

Chewing activity, when expressed as DM.kg/min, DM/bout and NDF.kg/min, was not affected by cottonseed or vitamin E. Nonetheless, when expressed as NDF/bout, it was higher for the animals fed cottonseed when compared to the control (0.210 vs. 0.152 kg) (Table 4.)

Table 4 - Effects of dietary cottonseed and vitamin E on cattle meal patterns

	Treatment				Probability	
	Control	CS	CSVitE	SEM	C1	C 2
Eating						
DM, (kg/min)	0,071	0,072	0,071	0,003	NS	NS
DM/bout, (kg)	2,326	2,386	2,630	0,179	NS	NS
NDF, (kg/min)	0,015	0,022	0,022	0,001	0,0001	NS
NDF/bout, (kg)	0,518	0,729	0,803	0,057	0,0479	0,0029
Ruminating						
DM, (kg/min)	0,050	0,036	0,036	0,003	0,0433	NS
DM/bout, (kg)	1,006	1,058	0,891	0,077	NS	NS
NDF, (kg/min)	0,011	0,011	0,011	0,001	NS	NS
NDF/bout, (kg)	0,224	0,323	0,272	0,022	0,0309	NS
Chewing						
DM, (kg/min)	0,028	0,024	0,023	0,001	0,0856	NS
DM/bout, (kg)	0,686	0,724	0,656	0,046	NS	NS
NDF, (kg/min)	0,006	0,007	0,007	0,001	NS	NS
NDF/bout, (kg)	0,152	0,221	0,200	0,014	0,0050	NS

DMI: dry matter intake; NDF: neutral detergent fiber; DM: dry matter; SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE).; NS: non-significant (p> 0,10).
Font: Nogueira, 2017.

3.3.3. Ruminal dynamics

Ruminal solid mass (11 vs. 8.3 kg) and ruminal total mass (62 vs. 57 kg) were greater for the animals fed cottonseed when compared to the control. When ruminal mass was expressed in relation to body weight, animals fed with cottonseed had 30% greater ruminal solid mass (12 vs. 9.7 g/kg) when compared to the control diet. Solid disappearance rate was lower when expressed as a percentage per hour (58 vs. 73 g/kg.hour) and greater when expressed as a kilogram per hour (0.66 vs. 0.60 g/kg), for animals fed cottonseed when compared to the control diet (Table 5).

Table 5 - Ruminal liquid, solid and total content, as well as solid disappearance rate of cattle fed cottonseed or vitamin E

	Treatments				Probability	
	Control	CS	CSVitE	EPM	C1	C2
RLM, (kg)	49,4	52,2	50,9	1,70	NS	NS
RSM, (kg)	8,29	10,8	11,2	0,45	0,0003	NS
RTM, (kg)	57,7	63,0	62,1	1,97	0,0490	NS
RLMBW,(g/kg)	57,7	59,5	58,4	1,60	NS	NS
RSMBW, (g/kg)	9,70	12,5	12,8	0,50	0,0009	NS
RTMBW, (kg)	67,4	72,0	71,3	1,90	NS	NS
RSD (g/kg/hour)	73.3	59.4	57.2	2.80	0.0068	NS
RSD, (kg/hour)	0.60	0.66	0.66	0.02	0.0030	NS

RLM: ruminal liquid mass, RSM: ruminal solid mass, RTM: ruminal total mass, RLMBW: ruminal liquid mass in relation to body weight, RSMBW: ruminal solid mass in relation to body weight, RTMBW: ruminal total mass in relation to body weight, RSD: ruminal solid disappearance rate; SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant ($p > 0.10$).
Font: Nogueira, 2017.

3.3.4. pH evaluation

Animals fed the cottonseed diet had higher mean (6.69 vs. 6.39), maximum (7,2 vs. 6.94) and minimum (6,15 vs. 5.83) ruminal pH levels than animals fed control diet. Animals fed cottonseed had lower pH time bellow 6,2 (84,3 vs. 410 min) when compared to the control.

Table 6. Ruminal pH of non-lactating animals fed dietary treatments.

	Treatments				P value	
	Control	CS	CSVitE	SEM	C1	C2
Ruminal pH						
Mean	6,39	6,77	6,62	0,07	0,0151	NS
Maximum	6,94	7,26	7,14	0,06	0,0112	NS
Minimum	5,83	6,26	6,05	0,09	0,0344	NS
pH< 5,8 (min)	128,3	0,00	51,7	43,0	NS	NS
pH< 6,0 (min)	213,3	0,00	81,7	53,6	NS	NS
pH< 6,2 (min)	410,0	26,7	142	68,1	0,0275	NS

SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
Font: Nogueira, 2017.

3.4. DISCUSSION

3.4.1. Nutrient intake, excretion and digestibility

The negative effects of fat supplementation on DMI have been reported in some (Harvatine and Allen, 2006; Martin et al. 2008) but not in all studies (Johnson et al. 2002; Moate et al. 2011). The consequences of fat supplementation can be: 1) DMI remains unaffected and GE intake increases, due to a higher energy density; 2) DMI is reduced, but GE intake is unchanged and 3) DMI is reduced to the extent that the GE intake is also reduced (Grainger and Beauchemin, 2011). DMI was not affected in the present study (Table 2), indicating that the fat concentration in the diet was within the nutritionally acceptable range. In addition, GE intake not changed (Table 2) due to replacement to NFC from EE.

The mechanisms of reduced DMI caused by lipid supplementation are related to the biohydrogenation process of unsaturated fatty acids in the rumen (NRC, 2001). Animals, fed the cottonseed diet, have a protected fat source within the seed coat, limiting its biohydrogenation via ruminal microbes (Baldwin and Allison, 1983). Whole oilseeds lessen the severity of digestive problems by encapsulation of fatty acids within their hard outer seed coat (Jenkins and Lundy, 2001). Then, it can be reasonable to assume that the cottonseed diets resulted in not enough free unsaturated fat within the

rumen and the ability of the microorganism to saturate the fatty acids wasn't exceeded. Hence, unsaturated fatty acids were not accumulated, resulting in a regular microbial digestion and DMI (NRC, 2001).

Sullivan et al. (2004) are in agreement with the present study, these authors working with lactating Holstein fed with diets containing cottonseed with 3% to 12% fatty acids and they observed similar DMI. Data reported by Oliveira et al. (2007b) prove benefits of protected fatty acids. The authors evaluated the effects of different dietary lipid sources (soybean grain with 62,0 g/kg and soybean oil with 55,4 g/kg EE in the total diet) on intake in buffalo bulls fed a high-concentrate diet. They observed that lipids inclusion caused a reduction of DMI when the energy source was soybean oil. However, when soybean grain was evaluated, not affect for the DMI was observed.

Forms of fat supplementation normally exert a negative effect on NDF digestibility to a different extent. The effect of fats on NDF digestibility depends on fat concentration, as well as type and nutrient composition of diets (Beauchemin et al. 2008). In the present experiment a protected lipid by seed was used and, according Patra et al. (2013), fat supplementation in the form of oil seeds has less negative effect on fiber digestibility than oil supplementation. In a meta-analyze study, these authors noted that NDF digestibility was affected by forms of fat supplementation (oil seeds vs. oils) and concluded that digestibility of NDF was not different (P=0,11) when oil seed is supplemented, but it was lower (P=0,02) when oil was added.

The effect of pH on fiber digestibility in the rumen has been extensively documented. Reduction in fiber digestion at low pH is likely the result of a reduction in the growth or activity of ruminal cellulolytic bacteria (Russell and Dombrowski, 1980, Grant and Mertens, 1992). According Strobel and Russel (1986), cellulose utilization decreased by 16% when the pH decreased from 6,7 to 6,0. The mean, minimum and maximum pH was higher than the control diet (Table 6) and it contributed to a better fiber digestibility.

In the present study, animals fed cottonseed remained less time in idles and more time in ruminating than the animal fed control diet (Fig. 1). Hence, this was enough to assist the negative lipid effect on fiber digestibility and DMI.

3.4.2. Feeding behavior

Cottonseed or vitamin E had no effect on eating rate (kg/min) and eating size (bouts/kg) (Table 4). In spite of cottonseed treatments had spent more time eating and be expected that these cows had lower intake in DM per minute, difference in the eating time (Table 3) was not enough to change the eating rate, when express as kg/min. Higher NDF intake (Table 2) in cottonseed treatments was responsible for higher NDF eating rate, when express as NDF.kg/min and NDF/bout.

As expected, ruminating rate, when express in DM.kg/min, was lower for the cottonseed treatments, once cows fed cottonseed spent more time ruminating than control (Table 3). In spite of cottonseed treatment had higher numbers of bout per day (Table 3), it would be expected that these cows had lower DM ruminated per bout, but the difference in the ruminating activities was not enough to change ruminating rate, when express as DM/bout.

With the higher NDF intake for the cottonseed treatments (Table 2) it would be expected these cows had a higher NDF ruminated and chewed per minute. However, cottonseed treatments spent more time ruminating and chewing (Table 3) and it canceled the difference among treatments for the NDF intake per minute. For the NDF/bout, as expected, cottonseed treatments had higher NDF ruminated and chewed per bout, once these animals had a higher NDF intake. In spite of cottonseed treatments had higher ruminating and chewing activity numbers it was not enough cancel out the higher NDF intake.

In spite of cottonseed treatments spent higher time chewing and had higher numbers of chewing over the day, it was not enough to change DMI per minute and DM per bout.

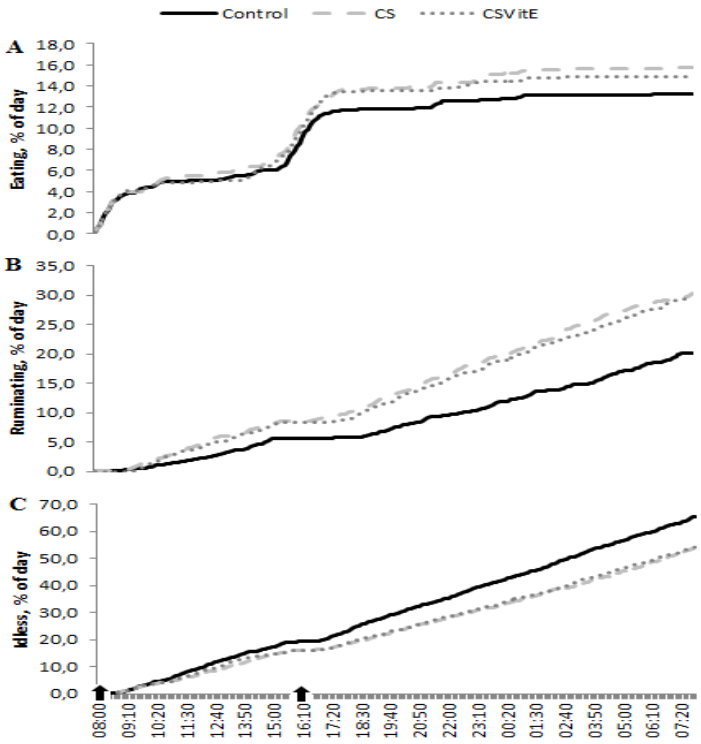
Animals have an occurring daily pattern of feed intake (DeVries et al. 2003b) with higher and lower levels of intake, rumination and idles across the day (Tolkamp et al. 2000). Regardless of treatments, the animals had the same behavior pattern, spending most of the time in idles, followed per rumination and eating (Fig. 1). Cottonseed addition increased total eating and ruminating time (Fig. 1), reflecting in the increase time to chewing and decrease idles time. The results are consistent with Iraira et al. (2013) who observed that heifers fed cottonseed had spent more time eating, ruminating and chewing than heifers fed barley straw. Clark and Armentano (1993) confirmed this,

when cottonseed was compared to alfalfa haylage in lactating dairy animals fed a diet with a 30:70 forage to concentrate ratio, both diets had similar feeding behavior.

Eating, ruminating and idles distribution patterns over 24 h were similar among treatments (Fig. 2). Eating total time over the day, 89%, 88% and 91% was performed during the diurnal period (06h00 to 18h00) for the control, CS and CSVitE, respectively. Ruminating activity was prevalent during the night period (18h00 to 06h00), corresponding to 70%, 61% and 62% of ruminating total time for the control, CS and CSVitE, respectively. Idles was well divided between day and night, once 54%, 54% and 54% total idles time was performed during the night for the control, CS and CSVitE, respectively (Fig. 2).

In fact, eating peaks occurred after fresh food was placed in front of the animals at 08h00 and 16h00 (Fig. 2), regardless of cottonseed or vitamin E inclusion, even if food remained in the manger from the previous feed. Total time eating, 36%, 31% and 32%, was spent in two hours, after feeding morning (08h00 to 10h00), for the control, CS and CSVitE respectively. Two hours after feeding afternoon (16h00 to 18h00), cows spent 29%, 28% and 31% of the total time eating for the control, CS and CSVitE. Thus, two hours after each feeding (08h00 to 10h00 and 16h00 to 1800) corresponded to 65%, 59% and 63% of the total time eating over the day for the control, CS and CSVitE, respectively. Our data are similar to those observed by Dürst et al. (1993). According to these authors, offering fresh feed is a strong stimulus for feeding, resulting in around 70% of the daily total proportion of intake being consumed immediately after offering.

Figure 2 - Cumulative time eating (A), ruminating (B) and in idles (C) over 24 hours for cattle fed cottonseed and vitamin E.



Fonte: Nogueira, 2017.

3.4.3. Ruminal dynamics

Ruminal solid and liquid mass data obtained in the present study are in agreement with Reynolds et al. (2004). These authors reported, on mean, ruminal solid mass of 8,5 kg with variations from 7,1 to 10,3 kg and liquid mass, on mean, 52,6 kg with variations from 48,9 to 57,7 kg. Patra et al. (2011) observed minimum liquid mass of 48,8 kg and maximum of 66,0 kg. For the ruminal solid mass, minimum was 4,9 kg and maximum was 11,4 kg, and for the total mass the minimum was 50,3 kg and maximum was 77,4 kg.

Higher solid mass and hence total mass in the animals fed with cottonseed likely is due to higher fiber content in their diets. Higher fiber content resulted in more ruminating time (Fig. 1). Thus, feed was kept more time inside the rumen, resulting in more solid mass.

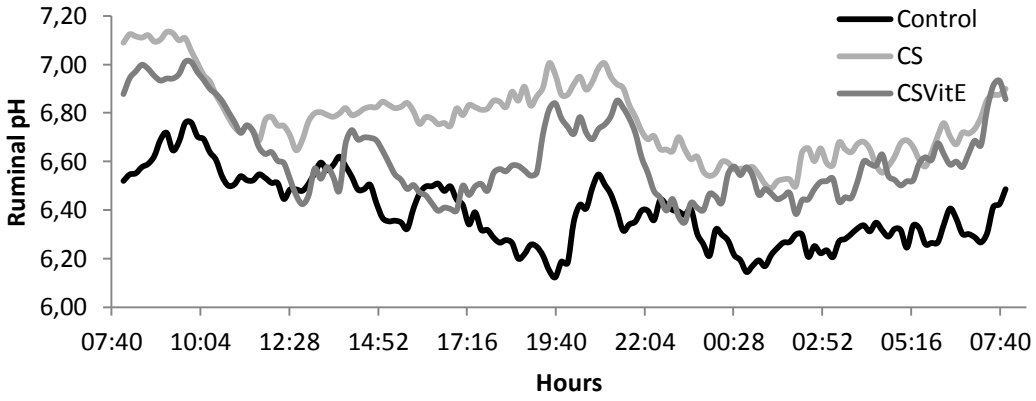
Ruminal solid disappearance rate, when express as kg/hour, was higher due higher ruminal solid mass inside the rumen of animals fed cottonseed compared to the

control. However, ruminal solid disappearance, when express as %/hour, was lower for the cottonseed treatments when compared to the control.

3.4.4. pH evaluation

In the present study, replacing from starch to fat explains the higher pH of cottonseed when compared to the control diet, as showed in the figure 3.

Figure 3 - Ruminal pH for diferentes dietary treatments over 24 hours.



Fonte: Nogueira, 2017.

Dietary content of NFC was reduced by 197 g/kg of DM in the cottonseed diets. Increased ruminal pH on CS and CSVitE reflected lesser NFC available in the rumen for the fermentation. In addition, the highest ruminal pH from the cottonseed diet may be partially explained by higher NDF content and it consequences on ruminating and chewing frequency. According Beauchemin et al. (2008), NDF is a stimulant for the ruminating and chewing, thus more saliva was produced and improvement in the buffer capacity in the rumen was observed.

3.5. CONCLUSION

Cottonseed is a recommended source of protected lipid for cattle when the objective is to provide high lipid concentrations in the diet. Their inclusion has positive consequences for the ingestive behavior and does not cause losses of DMI and fiber digestibility. Vitamin E is an additive that has no effect on the digestibility of nutrients and ingestive behavior of the animals, thus their use is not recommended due to the additional cost and absence of favorable results.

REFERENCES

ALLEN, M. S. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. **Journal of Dairy Science**, v. 83, p.1598-1624, 2000.

AOAC (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS). **Official methods of analysis**. Arlington, VA, Washington DC, 1980.

BATEMAN, H. G.; JENKINS, T. C. Influence of soybean oil in high fiber diets fed to nonlactating cows on ruminal unsaturated fatty acids and nutrient digestibility. **Journal of Dairy Science,** v. 81, p. 2451-2458, 1998.

BALDWIN, R. L.; ALLINSON, M. J. Rumen metabolism. **Journal of Animal Science.** v. 57, p. 461-477, 1983.

BEAUCHEMIN, K. A.; KREUZER, M.; O'MARA, F.; MCALLISTER, T. A. Nutritional management for enteric methane abatement: a review. **Australian Journal of Experimental Agriculture**, v. 48, p. 21-27, 2008.

BLOOMBERG, B. D.; HILTON, G. G.; HANGER, K. G.; RICHARDS, C. J.; MORGAN, J. B, VAN OVERBEKE, D. L. Effects of vitamin E on colour stability and palatability of strip loin steaks from cattle fed distillers grains. **Journal of Animal Science**, v. 89, p. 3769-3782, 2011.

BURGUER, J.; SAFINA, C.; GOCHFELD, M. Factors affecting vigilance in springbok: importance of vegetative cover, location in herd, and herd size. **Acta Ethologica**, v. 2, p. 97-104, 2000.

CAVALCANTI, M. C. A.; BATISTA, A. M. V.; GUIM, A.; LIRA, M. A.; RIBEIRO, V. L.; RIBEIRO NETO, A. C. Intake and ingestive behavior of sheep and goats fed with cactus pear (Opuntia ficus-indica Mill) and prickly pear (Opuntia sp.)**Acta Scientiarum:** Animal Sciences, v. 30, p. 173-179, 2008.

CHILIBROSTE, P.; TAMMINGA, S.; BOER, H.; GIBB, M. J.; DIKKEN, G. Duration of regrowth of ryegrass (Lolium perenne) effects on grazing behaviour, intake, rumen fill and fermentation of lactating cows. **Journal of Dairy Science**, v. 83, p. 984-995, 2000.

CLARK, P. W.; ARMENTANO, L. E. Effectiveness of neutral detergent fiber in whole cottonseed and dried distillers grains compared with alfalfa haylage. **Journal Dairy Science**, v. 76, p. 2644-2650, 1993.

CONCEIÇÃO, L. E. C.; MORAIS, S.; RONNESTAD, I. Tracers in fish larvae nutrition: a review of methods and applications. **Aquaculture**, v. 267, p. 62-75, 2007.

DEVRIES, T. J.; VON KEYSERLINGK, M. A. G.; WEARY, D. E.; BEAUCHEMIN, K. A. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. **Journal Dairy Science**, v. 86, p. 3354-3361, 2003.

DURST, B.; SENN, M.; LANGHANS, W. Eating patterns of lactating dairy-cows of 3 different breeds fed grass ad-lib. **Physiology and Behavior**, v. 54, p. 625-631, 1993.

FIRKINS, J. L.; ALLEN, M.; OLDICK, B.; ST-PIERRE, M. Modeling ruminal digestibility of carbohydrates and microbial protein flow to the duodenum**. Journal Dairy Science**, v. 81, p. 3350-3369, 1998.

FISHER, V.; DESWYSEN, A. G.; DESPRES, L.; DUTILLEU, P.; LOBATO, J. F. P. Ingestive behavior of sheep fed a hay-based diet over a six-month period. **Revista Brasileira de Zootecnia**, v. 26, p. 1032-1038, 1997.

GIRARD, J. Metabolic adaptations to change of nutrition at birth. **Biology of the Neonate**, v. 58, p. 3-15, 1990. Supplement 1.

GRAINGER, C.; BEAUCHEMIN, K. A. Can enteric methane emissions from ruminants be lowered without lowering their production? **Animal Feed Science and Technology**, v. 167, p. 308-320, 2011.

GRANT, R. H.; MERTENS, D. R. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. **Journal Dairy Science**, v. 75, p. 2762–2768, 1992.

HARVATINE, K J.; ALLEN, M. S. Effects of fatty acid supplements on feed intake, and feeding and chewing behavior of lactating dairy cows. **Journal Dairy Science**. v. 89, p. 1104-1112, 2006a.

IRAIRA, S.P.; RUIZ DE LA TORRE, J.L, RODRIGUES.; PRADO, M.; CALSAMIGLIA, S.; MANTECA, X.; FERRET, A. Feed intake, ruminal fermentation, and animal behavior of beef heifers fed forage free diets containing nonforage fiber sources. **Journal Animal Science**, v. 91, p. 3827-3835, 2013.

JENKINS, T.; LUNDRY, F. Feeding various fat sources to lactating dairy cows and their effects on milk quality. 31-38. Available from: <http://extension.psu.edu/animals/import/dairy/nutrition/pdf/jenkins-fat-sources-and-effects on-milkquality-2001.pdf, 2001>. Access: 28 July 2106.

JOHNSON, K. A.; KINCAID, R. L.; WESTBERG, H. H.; GASKINS, C. T.; LAMB, B. K.; CRONRATH, J. D. The effect of oilseeds in diets of lactating cows on milk production and methane emissions. **Journal of Dairy Science**, v. 85, p. 1509-1515, 2002.

JORGE, J. R. V.; ZEOULA, L. M.; PRADO, I. N.; SILVA, R. R, ANDRADE, R. V.; PRADO, J.; BUBLITZ, E. E. Lipids in diets for Holstein steers: apparent digestibility. **Revista Brasileira de Saúde e Produção Animal,** v. 9, p. 743-753, 2008.

MAEKAWA, M.; BEAUCHEMIN, K. A.; CHRISTENSEN, D. A. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. **Journal of Dairy Science**, v. 85, p. 1165-1175, 2002.

MARTIN, C.; ROUEL, J.; JOUANY ,J. P.; DOREAU, M.; CHILLIARD, Y. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. **Journal of Animal Science**, v. 86, p. 2642-2650, 2008.

MOATE, P. J.; WILLIAMS, S. R. O.; GRAINGER, C.; HANNAH, M. C.; PONNAMPALAM, E. N.; ECKARD, R. J. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. **Animal Feed Science and Technology**. v. 167, p. 254-264, 2011.

NATIONAL RESEARCH COUNCIL. **Nutrient requeriments of dairy cattle.** Washington, DC: National Academy of Sciences, 2001.

OLIVEIRA, R. L.; ASSUMÇÃO, D. M. P.; BARBOSA, M. A. A. F.; LADEIRA, M. M.; SILVA, M. M. P.; MASCARENHAS, A. G.; SNEL-OLIVERIA, M. V. Effect of different fat sources on intake, digestibility and blood urea nitrogen of feedlot water buffalo steers. **Revista Brasileira de Zootecnia**, v. 36, p. 733-738, 2007b.

PATRA, A. K. Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. **Asian Journal of Animal and Veterinary Advances,** v. 6, p. 416-428, 2011.

PATRA, A. K. The effect of dietary fats on methane emissions, and its other effect on digestibility, rumen fermentation and lactation performance in cattle: a meta-analysis. **Livestock Science,** v.155, n.2/3, p. 244-254, 2013.

REYNOLDS, C. K, DURST, B.; LUPOLI, B.; HUMPHRIES, D. J.; BEEVER, D.E . Visceral tissue mass and rumen volume in dairy cows during the transition period from late gestation to early lactation**. Journal of Dairy Science**, v. 87, p. 961-971, 2004.

ROCHA NETO, A. L.; VELOSO, C. M.; SILVA, F. F.; SOUZA, D. R.; COSTA, L. T.; MURTA, R. M.; SILVA, R. R.; SILVA, J. C. P. M.; SOUZA, D. D.; MENESES, M. A. ngestive behavior of dairy cows fed sugar cane or cassava aerial portion hay. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 64, p. 1629-1638, 2012.

RUSSEL, J. B.; DOMBROSKI, D. B. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. **Applied and Environmental Microbiology,** v. 39, p. 604-610, 1980.

STROBEL, H. J.; RUSSEL, J. B. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. **Journal of Dairy Science,** v. 69, p. 2941-2947, 1986.

SULLIVAN, H. M.; BERNARD, J. K.; AMOS, H. E.; JENKINS, T. C. Performance of lactating dairy cows fed whole cottonseed with elevated concentrations of free fatty acids in the oil. **Journal of Dairy Science**, v. 87, p. 665-671, 2004.

TOLKAMP, B. J.; SCHWEITZER, D. P. N.; KYRIAZAKIS, I. The biologically relevant unit for the analysis of short-term feeding behavior of dairy cows. **Journal of Dairy Science**, v. 83, p. 2057-2068, 2000.

VAN SOEST, P. J.; ROBERTSON, G. B.; LEWIS, B.A. Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. **Journal of Dairy Science,** v. 74, p. 3583-3597, 1991.

VAN SOEST, P. J. **Nutritional ecology of the ruminant**. 2nd ed. Ithaca: Cornell University Press, 1994.

ZARRYS, P. I.; HOGAN, S. A.; O'SULLIVAN, M.G.; ALLEN, P.; KERRY, J. P. Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. **Meat Science**, v. 79, p. 648-655, 2008.

4. METHANE MITIGATION AND CHANGES IN THE RUMINAL FERMENTATION AND ENERGY RELEASE IN THE GASTROINTESTINAL TRACT OF COWS FED COTTONSEED AND VITAMIN E

Abstract: Methane (CH_4) production by ruminants is a consequence of the digestive process and it represents energy losses for the animal. Lipids are used to improve diet energy content. High lipids level can have undesirable consequences for ruminant and their products. Antioxidant sources can cancel out or minimize these effects. Thus, evaluation of ruminal parameters, such as methane and short chain fatty acids (SCFA) production, in addition to energy release in gastrointestinal tract caused by different diets, are valuable information. Six cannulated cows were distributed in a replicate 3x3 Latin square. Treatments were: 1) control diet; 2) CS: basal diet plus 30% cottonseed and 3) CSVitE: basal diet plus 30% of cottonseed plus 500 UI of vitamin E. Results were compared through orthogonal contrast. Methane, SCFA and ammonia nitrogen ($N\text{-}NH_3$) were analyzed using *ex situ* ruminal fermentation technique, in addition to estimative of energy release in the gastrointestinal tract. Cottonseed inclusion reduced enteric methane emissions in 42,3% compared to the control. Ruminal reduction in the production of acetate was 33,8%, butyrate was 47,2%, total SCFA was 29,1% and the acetate propionate production ratio was 36,1% for cottonseed treatments compared to the control. Energy release in the rumen (Mcal/ani/d) as methane and butyrate was reduced to 26,2% and 30% respectively. On the other hand, propionate and intestine energy release (Mcal/ani/d) was increased in 42,8% and 50,3% for cottonseed treatments compared to the control. Including 30% of cottonseed in cattle diet reduces rumen fermentation and changes the place where energy is released. Cottonseed is a nutritional strategy to mitigate methane; furthermore, it has positive effects on SCFA production. The use of vitamin E did not result in improvements in ruminal fermentation, thus so their use is unfeasible.

Keywords: Enteric methane, short chain fatty acids, lipids, antioxidant.

4.1. INTRODUCTION

Enteric methane emissions from cattle supply chains are problematic with respect to the energy utilization efficiency of feed. According to Buddle et al. (2011), 5% to 9% of gross energy consumed by animals is lost as methane, in addition there is

an environmental impact due to such emissions According to Geber et al. (2013), cattle are responsible for 14.5% of anthropogenic GHG´s and 25% of the methane emissions from human activities.

A big challenge in the ruminant production system is to develop diets and management systems that minimize methane emissions in order to allow lower energy losses and then, improve feed efficiency and animal productivity (Nardone et al. 2010).

The addition of lipids in the diet is an option recognized to reduce enteric methane emissions. The influence of lipids in methane emissions, digestibility and rumen fermentation vary between studies, which may be associated with the type and concentration of fatty acids in the diet (Grainger & Beauchenin, 2011).

Cottonseed is often used in ruminant feed by presenting a high concentration of oil, protein and fiber. Few foods can gather these nutrients in high concentrations and present high fiber degradability such as cottonseed (Pesce, 2008).

The inclusion of lipids can have their use limited by the possibility of unsaturated fatty acids suffer lipid peroxidation, mainly with the peroxidation of cattle products, as meat and milk, and can accelerate myoglobin oxidation, with losses of physical, chemical and sensory characteristics (Zakrys et al. 2008).

A main way to reduce or prevent lipid peroxidation is through the use of antioxidants, and generally substances or conditions with preventive oxidation properties. In recent years, supplemental vitamin E in the diet of ruminants has been studied for its potential role in preventing lipid peroxidation, giving stability to lipid deposits and improving the resistance to oxidation (Bloomberg et al. 2011).

Rumen environment is relatively free of oxygen and suitable for the colonization and growth of rumen microbes which are anaerobes. However, a small amount of oxygen, which is harmful to rumen microbes, may go into the rumen with saliva, feeds, and drinking water and diffuses from blood into rumen (Wey et al., 2015). Therefore, supplementing vitamin E to relieve oxidation effects from free oxygen could be beneficial to rumen microbes and consequently feed digestion. In in vitro rumen fermentation, Hou et al. (2013) reported that supplementing vitamin E at 2 mg/80 mL incubation liquid increased in vitro rumen acetate and total SCFA production and decreased butyrate production.

The objective of this study was to evaluate the effect high level of cottonseed inclusion and the presence or absence of vitamin E on methane and SCFA production, as well as evaluate the energy release in the gastrointestinal tract of cows.

4.2. MATERIAL AND METHODS

4.2.1. Study location and ethical issue

The study was conducted at the University of São Paulo, Pirassununga, Brazil. The experiment was approved by and complied with the guidelines set out by the Ethics Committee in the Use of Animals of the University of São Paulo, under application number nº 009/2013, in respect to animal experimentation and care of animals used for scientific purposes.

4.2.2. Animals, housing and feeding

Six Holstein dairy cows, not pregnant and non-lactating with rumen canula and average body weight of 876 kg (±16,1), were arranged in individual pens with free access to water and sand bedded. Cows were fed ad libitum twice daily (0800h and 1600h). The feed was weighed daily and offered to each animal after the feed residue from the previous day had been removed. The vitamin E amount was weighted daily to offer 500 IU per animal per day, and it was offered combined with the concentrate. Vitamin E was weighed daily according to the amount of dry matter that would be offered. This was mixed and homogenized in the concentrate and offered to the animals.

4.2.3. Experimental design and treatments

Replicated 3 x 3 Latin squared design with 3 periods was used. Three dietary treatments were as follows: 1) control diet, 2) CS: cottonseed diet and 3) CSVitE: cottonseed diet plus vitamin E diet. The fatty acids and cottonseed level in the diet was established according to Andrade et al. (2010). The vitamin E level was established

according Baldwin et al. (2010) and Montgomery et al. (2005). The vitamin E source was Lutavit E 50 BASF, with 50% alpha tocopheryl acetate. Vitamin E was weighed daily according to the amount of dry matter that would be offered. This was mixed and homogenized in the concentrate and offered to the animals.

The ingredient and chemical composition of the experimental diets are presented in Table 1.

4.2.4. Sampling Schedule

The trial consisted of three experimental periods; each one lasted 21 days. The first 10 days of each period were used to diet adaptation. The days between the 11th to the 15th were used to measure feed intake. The days between the 11th to the 17th were used to measure DM digestibility and collect feces. The 18th day was used to collect rumen content. The 20th to 21th days the rumen was emptied and the solid mass was evaluated.

4.2.5. Feed intake

Feed intake was determined during the 11th to 15th days of each period by weighing feeds offered to and refused by the cows. Refusal was recorded once daily and the feeding rate was adjusted to yield orts on the basis of at least 10% of the amount supplied (on an as-fed basis)

4.2.6. Ruminal empting

The last 2 days of each period, before morning feeding and 3 hours after morning feeding respectively, ruminal contents were manually removed according Chilibroste et al. (2000). Using a strainer, ruminal contents were separated in solid and

liquid content, and then they were weighted and sampled. Immediately after that, ruminal contents were replaced back in the rumen. The solid and liquid samples were dried at 60°C (forced-air oven) for 72 hours, and dry matter content was determined. The solid and liquid mass was calculated using solid and liquid content weighted and adjusted by dry matter content.

4.2.7. Dry matter digestibility and fecal output

Dry matter digestibility and fecal output were determined using chromium oxide. During days 11th until 17th, 15 g/head/day of indigestible marker was placed twice daily (800h and 1600h before feeding) via rumen fistula. Feces were manually collected twice per day via the rectum from the 14th until 18th at 800h and 1600h after feeding. A composite of 200g samples were then analyzed for chromium oxide concentration according to Conceição et al. (2007). DM digestibility, expressed as percentage, was calculated using the chromium concentration in the diet and chromium concentration in the feces using the equation:

$$Digestibility = 100 - (\frac{chromiun\ in\ the\ diet \div DMI \times 100}{chromiun\ in\ the\ feces}) \times 100$$

Fecal output, expressed as kg (DM basis), was calculated as:

$$Fecal\ output = \frac{(100\ - DM\ digestibility) \times DMI}{100}$$

4.2.8. Methane and SCFA production

Ruminal contents samples were collected on 18th day of each period through the ruminal cannula at 0, 3, 6, 9 and 12 h after the morning meal. On this day, animals were fed once in the morning. The evening meal was offered only after the collection of

the 12 h sample. Approximately 300mL of rumen fluid (using a motorized vacuum pump) and 300g of solid content (with hands) were collected, at each sampling time from three different parts of the rumen (dorsal sac in the front, middle and back).

The two fractions were mixed in the proportions of 66% liquid phase and 33% solid phase and homogenized before preparation for analysis of SCFA, CH_4 and NH_3-N with the *ex situ* ruminal fermentation technique (Rodrigues et al., 2012).

Each time, four bottles were prepared; two bottles were used for incubation (T_{30}) and two of them were used as a blank (T_0). The mixed rumen contents (30 mL) were pressed through a funnel into a 50 mL capacity bottles. After that, the bottles were capped with rubber corks and sealed with an aluminum seal. Then the bottles were washed with CO_2 by means of needles for input and output to ensure an anaerobic environment. After 30 minutes incubation, fermentations were blocked by autoclaving (pressure and temperature for 15 minutes).

The measurements of total gas volume produced in incubated (T_{30}) and not incubated bottles (T_0) was done using a pressure transducer (Datalogger Universal® - logger model AG5000) connected to a syringe with a needle. The gas volume was obtained by the sum between the volume obtained at the transducer plus the head space. The determination of CH_4 concentration was performed by gas chromatography (Thermo Scientific®, Schimadzu model - GC), injecting 0.5 mL of gas from each bottle, according to Kaminski *et al.* (2003) in a controlled temperature environment (25°C).

The volume of liquid within the incubated (T_{30}) and not incubated (T_0) bottles was calculated as the difference between the weight of the bottle sample after drying in an oven with forced air circulation at 105°C for 24 hours and the weight of the bottles before the oven. The solid content of the bottles was obtained by the weight difference between the bottle containing the sample after drying in oven and the weight of the empty bottle (before filling with ruminal content sample). For SCFA analyses, that included acetate, propionate and butyrate, a fraction of ruminal fluid from each bottle was centrifuged at 2,000 × g for 20 min, and 2.0 mL of the supernatant was added to 0.4 mL of formic acid and frozen at –20°C for further analyses, according to Erwin et al. (1961). SCFA were measured by gas chromatography (Focus GC, Thermo Scientific®, West Palm Beach, FL) using a glass column with 1.22 m in length and 0.63 cm in diameter packed with 80/120 Carbopack B-DA/4% (Supelco, Sigma-Aldrich®, St. Louis, MO).

The quantification of CH_4 production was obtained by multiplying the total volume of gas (mL) and the CH_4 concentration in the gas phase (mmol/mL) obtained in the incubated bottle (T_{30}), and then the result was subtracted from the value that was produced in the bottle not incubated (T_0). The individual quantification of SCFA was obtained by multiplying the liquid volume (mL) and the concentration of each SCFA (mmol/mL) obtained in the incubated bottle (T_{30}), and this value was also subtracted from that obtained on the bottle not incubated (T_0). CH_4 (2) or SCFA (3) production was obtained by the following formula:

$$\text{CH4Prod.} = (methane\ con. \times Total\ Gas\ Vol.)T_{30} - (CH4\ con. \times Total\ Gas\ Vol.)T_0$$

Where:

CH_4 Prod.: methane production at the moment between ruminal content injection in the bottle and inactivation

methane Con.: methane Concentration (mmol/mL)

Total Gas Vol.: total gas volume was obtained by the sum between the volume obtained at the transducer plus the head space (mL)

T_{30}: incubation time of 30 min;

T_0: incubation time of 0 min (not incubated).

$$\text{SFCA Prod.} = (SCFA\ con. \times Total\ Liq.Vol.)T_{30} - (SCFA\ con. \times Total\ Liq.Vol.)T_0$$

Where:

SCFA Prod.: SCFA production at the moment between ruminal content injection in bottle and inactivation

SCFA Con.: SCFA concentration (mmol/mL)

Total Liq. Vol.: total liquid volume at penicillin bottle obtained by weight difference between before and after the oven (mL)

T_{30}: incubation time of 30 min

T_0 = incubation time of 0 min (not incubated).

Thereafter, CH_4 and SCFA production were expressed based on the solid content of the bottles (grams or kilograms).

After quantification of the fermentation products (CH_4 and SCFA) of the sample contained in each bottle, each product was multiplied by its combustion heat in order to express the CH_4 production in relation to the energy from the produced fermentation. Thus, the relative energy loss (REL) was the ratio between the energy in the methane produced and the energy sum in all the quantified fermentation products (CH_4 and SCFA), expressed as a percentage. For this, literature data was used assuming that acetic, propionic, butyric, CH_4 and CO_2 had 3,49, 4,98, 5,96, 13,16 and 0.0 kcal per gram or 209,40, 368,52, 524,48, 210,56 and 0.0 kcal per mol, respectively. The relative energy loss was calculated using the formula:

$$\mathrm{REL} = \frac{\text{Ɛ}CH_4}{\text{Ɛ}CH_4+\text{Ɛ}C_2+\text{Ɛ}C_3+\text{Ɛ}C_4}$$

Where:

REL.: relative energy loss (%)

$\text{Ɛ}CH_4$: methane energy (kcal/g or kcal/mol)

$\text{Ɛ}C_2$: acetic acid energy (kcal/g or kcal/mol)

$\text{Ɛ}C_3$: propionic acid energy (kcal/g or kcal/mol)

$\text{Ɛ}C_4$: butyric acid energy (kcal/g or kcal/mol)

4.2.9. Ammonia nitrogen concentration and balance

For NH_3-N concentration determination, 2.0 mL of centrifuged sample of each bottle, after microbial inactivation, was mixed with 1 mL of 1 N of H_2SO_4 solution, and the tubes were immediately frozen until the colorimetric analyses, according to the method described by Kulasek (1972) and adapted by Foldager (1977). The balance was obtained subtracting NH_3-N concentration after 30 min of incubation (T_{30}) from the baseline (T_0). With this procedure it is possible to evaluate whether the balance of ammonia production in the rumen is positive or negative. In this paper, this information was expressed in terms of changes in concentration (mg/dL) per hour.

4.2.10. Energy release estimative

Gross energy intake (Mcal/ani/d) was calculated by multiplication of DMI (kg) and diet gross energy (Mcal/kg). To calculate the energy release of acetate, propionate, butyrate and methane (Mcal/ani/d) in the rumen, these metabolite productions (g/kg/d) were respectively multiplying by combustion heat (Mcal/g), and then multiplying by ruminal solid mass (kg).

Energy release in the rumen, when expressed in terms of %GEI or %DE, was obtained dividing acetate, propionate, butyrate and methane release (Mcal/ani/d) by gross energy intake (Mcal/ani/d) or digestive energy (Mcal/ani/d) and then, multiplying by 100.

Methane release in the cecum and colon (C&C) was considered as 5% of total methane release. According to Dini et al. (2012), enteric methane is produced mainly in the rumen (95%) and, to a smaller extent (5%), in the large intestine.

Energy release in the intestine (Mcal/ani/d) was calculated from gross energy intake (Mcal/ani/d) subtracting from acetate, propionate, butyrate and methane release in the rumen (Mcal/ani/d) plus feces gross energy (Mcal/ani/d) and methane release in the cecum and colon (Mcal/ani/d), following the equation:

$$\mathrm{ERI} = \mathrm{GEI} - (\mathrm{C2} + \mathrm{C3} + \mathrm{C4} + \text{feces GE} + \mathrm{C\&C\ methane})$$

Where:

ERI: energy release in the intestine (Mcal/ani/d)

GEI: gross energy intake (Mcal/ani/d)

C2: acetic (Mcal/ani/d)

C3: propionic (Mcal/ani/d)

C4: butyric (Mcal/ani/d)

feces GE: energy release in the feces (Mcal/ani/d)

C&Cmethane: methane release in cecum and colon (Mcal/ani/day).

Energy release in the intestine, expressed in terms of %GE or %DE, was obtained dividing energy release in the intestine (Mcal/ani/d) by GEI (Mcal/ani/d) or DE (Mcal/ani/d) and then, multiplying by 100.

Energy release in feces, expressed in terms of %GEI, was obtained dividing feces energy content (Mcal/ani/d) by gross energy intake (Mcal/ani/d) and then, multiplying by 100.

4.2.11. Laboratory analysis

Briefly, pooled feed ingredients, as well feces samples, were collected and stored at −20 °C. Samples were dried at 60°C during 48 hours and milled through a one-mm screen using a Willey mill. The DM content was determined at 100°C for 4 h followed by cold weighing (method 930.15, AOAC, 1995). Nitrogen content was determined by the micro Kjeldahl method (AOAC, 1995) and then, it was multiplied by 6.25 to determine CP. EE was determined using light petroleum ether in the Soxhlet apparatus (method 920.39, AOAC, 1995). GE was determined by combustion using an adiabatic calorimeter bomb according to AOAC (1995). NDF, ADF and lignin were determined using the sequential method with Ankom® Filter Bag technique and heat stable α−amylase (method 973.18, AOAC, 1995).

4.2.12. Statistical analysis

The data were analyzed using the MIXED procedure of SAS (Statistical Analysis System, version 9.0). Cows, in each period, were considered the experimental units. Before the actual analysis, the data were analyzed for the presence of disparate information ("outliers") and the normality of residuals (Shapiro-Wilk). Individual observation was considered outlier when standard deviations in relation to mean was bigger than +3 or lesser than -3.

For the ruminal solid mass, gross energy intake and energy release data, the model used included the fixed effect of treatment and random effect square, period, and animals within the square. These variables were analyzed using the following model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + A_l(S_k) + e_{ijkl}$$

Where:

$Y_{ijkl:}$ the dependent response variable

μ: the overall mean

$T_{i:}$ treatment effect

$P_{j:}$ period effect

$S_{k:}$ square effect

$A_l(S_k)$: animals within square effect

$e_{ijkl:}$ the residual error term.

For the methane, SCFA and ammonia variables, data were analyzed using mixed models (PROC MIXED). Among 15 different covariance structures tested, the selected model was chosen based on the lower value of Corrected Akaike Information Criterion (AICC) (Wang and Goonewardene, 2004). In this model, the treatment, time and interaction treatment*time effects were considered fixed, and the effects of period, square and animal within square were considered random. These variables were analyzed using the following model:

$$Y_{ijklm} = \mu + T_i + P_j + S_k + A_l(S_k) + TI + (T_i \times TI)_{ij} + e_{ijklm}.$$

Where:

Y_{ijklm}: the dependent response variable

u: the overall mean

T_i: treatment effect

P_j: period effect

S_k: square effect

$A_l(S_k)$: cows within square effect

$TI_{:}$ time effect

$(T_i \times TI)_{ij}$: interaction effect

e_{ijklm} = the residual error term.

Non-significant (NS) was considered when P value was higher than 10%.

Contrast statements were used to evaluate differences between means: C1) Control vs. CS and CSVitE and C2) CS vs. CSVitE. Statistical significance was declared at $P \leq 0.05$.

4.3. RESULTS

4.3.1. Ruminal parameters

No effect was observed for the ammonia nitrogen in the 0 and 30 min. To the balance, in hours, animals feed vitamin E had a lower (0,85 vs. 1,99 mg/dL.hours) ammonia nitrogen balance compared to the animals feed without vitamin E (Table 7).

Inclusion of cottonseed in the diet, regardless of vitamin E, decreased acetate and butyrate production when compared to the control diet. The propionate production was similar among treatments. Additionally, SCFA total production and acetate:propionate ratio was reduced by cottonseed supplementation compared to the control diet (Table 7).

Feeding cows with cottonseed, regardless of vitamin E, decreased the methane production when compared to the control diet.

Including cottonseed or vitamin E in diet did not affect the relative energy loss (REL) (Table 7).

Table 7. Ruminal fermentation of non-lactating cows fed dietary treatments.

	Treatments				Probability			
	Control	CS	CSVitE	SEM	C1	C2	Time	T x Ti
N-NH3, mg/dL/hour								
T_0	22,45	21,95	21,37	0,863	NS	NS	0,001	0,0010
T_{30}	23,56	23,94	22,22	0,934	NS	NS	0,001	0,0010
Balance, hours	1,110	1,990	0,850	0,413	NS	0,0004	NS	NS
Acetate								
T_0, mmol/L	71,13	67,23	67,12	0,897	NS	NS	0,0110	NS

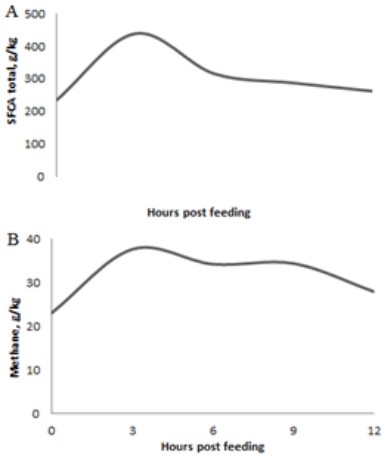
T_{30}, mmol/L	76,11	71,35	71,05	0,999	0,0211	NS	0,0009	NS
mmol/g DM/h	0,146	0,099	0,093	0,008	0,0028	NS	0,0187	NS
mol/kg DM/d	3,502	2,398	2,232	0,197	0,0028	NS	0,0187	NS
g/kg DM/d	210,1	143,9	133,9	11,87	0,0028	NS	0,0187	NS
EB, Mcal/kg/d	0,733	0,502	0,467	0,041	0,0028	NS	0,0187	NS
Propionate								
T_0, mmol/L	15,25	20,75	20,94	0,499	0,0010	NS	0,0001	NS
T_{30}, mmol/L	16,80	22,74	22,92	0,553	0,0016	NS	0,0001	NS
mmol/g DM/h	0,045	0,048	0,046	0,003	NS	NS	0,0101	NS
mol/kg DM/d	1,093	1,153	1,108	0,074	NS	NS	0,0101	NS
g/kg DM/d	80,94	85,35	82,01	5,514	NS	NS	0,0101	NS
EB, Mcal/kg/d	0,403	0,425	0,408	0,027	NS	NS	0,0101	NS
Butyrate								
T_0, mmol/L	12,71	9,346	9,129	0,293	0,0001	NS	0,0591	0,0756
T_{30}, mmol/L	14,20	10,35	10,03	0,324	0,0001	NS	0,0063	NS
mmol/g DM/h	0,042	0,024	0,020	0,002	0,0001	NS	0,0194	NS
mol/kg DM/d	1,030	0,583	0,503	0,048	0,0001	NS	0,0194	NS
g/kg DM/d	90,72	51,36	44,30	4,228	0,0001	NS	0,0194	NS
EB, Mcal/kg/d	0,540	0,306	0,264	0,025	0,0001	NS	0,0194	NS
SCFA total								
T_0, mmol/L	99,09	97,34	97,19	13,30	NS	NS	0,0048	0,0938
T_{30}, mmol/L	107,1	104,4	104,0	14,69	NS	NS	0,0010	NS
mmol/g DM/h	0,234	0,172	0,160	0,012	0,0054	NS	NS	NS
mol/kg DM/d	5,627	4,135	3,844	0,293	0,0054	NS	NS	NS
g/kg DM/d	381,8	280,6	260,2	19,60	0,0054	NS	NS	NS
EB, Mcal/kg/d	1,677	1,233	1,139	0,084	0,0054	NS	NS	NS
C2:C3 ratio								
Concentration	4,766	3,294	3,349	0,098	0,0002	NS	0,0005	NS
Production	3,607	2,193	2,413	0,172	0,0097	NS	0,0617	NS
Methane								
T_0, mmol/bottle	0,039	0,0263	0,028	0,001	0,0001	NS	NS	NS
T_{30}, mmol/bottle	0,152	0,104	0,097	0,004	0,0003	NS	0,0001	NS
mmol/g/h	0,114	0,069	0,063	0,003	0,0001	NS	0,0001	NS
mol/kg/d	2,737	1,650	1,507	0,088	0,0001	NS	0,0001	NS
g/kg/d	43,79	26,40	24,13	1,420	0,0001	NS	0,0001	NS
EB, Mcal/kg/d	0,576	0,347	0,317	0,018	0,0001	NS	0,0001	NS
REL, %	31,32	29,67	27,35	1,368	NS	NS	NS	NS

SCFA: short chain fatty acids; C2:C3 ratio: acetate to propionate ratio REL: relative energy loss; SEM: stand error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); TxTI: interaction treatment time; NS: non-significant (p> 0,10).
Font: Nogueira, 2017.

No time x treatment interaction was observed among the variables, but time effect was significant ($p<0.05$). On average, SCFA total production was 234, 439, 315, 287 and 262 g/kg DM to 0, 3, 6, 9 and 12 hours post feeding respectively. On average,

methane production was 23, 38, 34, 34 and 27 g/kg DM to 0, 3, 6, 9 and 12 hours post feeding (Fig.3)

Figure 3**.** Average SCFA total (fig. A) and methane (fig. B) production (g/kg DM) in hours post feeding.



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4.3.2. Energy partition

No difference was observed for the dry matter intake, dry matter digestibility or dry matter excretion. Cottonseed inclusion, regardless of VitE had 32% higher ruminal solid mass when compared to the control treatment (Table 8).

Propionate and intestine energy release when expressed as Mcal/ani/d were respectively 43% and 57% higher for the cows fed cottonseed when compared to the control diet. Butyrate and methane energy release when expressed as Mcal/ani/d were respectively 32% and 26% lower for the cottonseed treatments when compared to the control treatment. Acetate and feces energy release when expressed as Mcal/ani/d were similar among treatments (Table 8).

Butyrate released when expressed as %GE (5,4% vs. 9,2%) and %DE (8,0% vs. 14%) was lower for the cottonseed treatments when compared to the control. Methane released when express as %GE (6,2% vs. 9,7%) and %DE (9,3% vs 15%) was lower for the cottonseed treatments when compared to the control. In the intestine the energy released when express as %GE (37,5% vs. 27,3%) and %DE (53,2% vs. 39%) was higher for the cottonseed treatments when compared to the control. Acetate and

propionate energy released when express as %GE and %DE were similar among treatments (Table 8).

Table 8. Estimative of energy release in the gastrointestinal tract of cows feed different diets.

	Treatments				Probability	
	Control	CS	CSVitE	SEM	C1	C2
Dry matter intake, kg	14,6	15,4	15,4	0,61	0,0649	NS
Dry matter digestibility, g/kg	676	679	677	22,4	NS	NS
Dry matter excretion, kg	3,67	4,02	4,06	0,19	NS	NS
Ruminal solid mass, kg	8,29	10,8	11,2	0,45	0,0003	NS
Gross energy intake, Mcal/ani/d	50,5	58,7	58,6	3,12	NS	NS
Energy release in the rumen						
Acetate						
Mcal/ani/d	5,88	5,46	4,98	0,28	NS	NS
GE, %	11,9	9,88	9,39	0,96	NS	NS
DE, %	18,8	15,0	14,4	1,98	NS	NS
Propionate						
Mcal/ani/d	3,20	4,61	4,53	0,30	0,0343	NS
GE, %	6,40	8,05	8,09	0,55	NS	NS
DE, %	9,82	12,2	12,2	1,06	NS	NS
Butyrate						
Mcal/ani/d	4,59	3,33	2,92	0,24	0,0033	NS
GE, %	9,22	5,78	5,07	0,51	0,0002	NS
DE, %	14,2	8,60	7,58	0,93	0,0021	NS
Total SCFA						
Mcal/ani/d	13,6	13,4	12,4	0,51	NS	NS
GE, %	27,5	23,7	22,5	1,59	NS	NS
DE, %	42,8	35,9	34,2	3,37	NS	NS
Methane						
Mcal/ani/d	4,91	3,76	3,48	0,27	0,0013	NS
GE, %	9,72	5,96	6,59	0,50	0,0005	NS
DE, %	15,0	9,87	8,73	0,93	0,0031	0,0031
Energy release in the intestine						
Mcal/ani/d	14,9	22,5	24,4	2,90	0,0405	NS
GE, %	27,3	36,1	38,9	3,61	0,0239	NS
DE, %	39,0	51,8	54,7	4,14	0,0161	NS
Energy release in the Feces						
Mcal/ani/d	16,0	18,1	17,4	1,03	NS	NS
GE, %	33,3	31,9	31,0	2,33	NS	NS

GE: gross energy; ani: animal; DE: digestible energy; SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
Font: Nogueira, 2017

4.4. DISCUSSION

4.4.1. Ruminal parameters

According to Doreau and Ferlay (1995), the ammonia concentration in the rumen is the result of nitrogen balance between entry sources (degradable feed N and N recycling) and outputs (incorporation into microbes, N absorption and ammonia N outflow). T_0 correspond to ruminal N-NH_3 concentration. In the present study, ruminal ammonia concentration was, on average, of 21.8 mg/dL. This concentration is suitable for the ruminal fermentation. According to Leng (1990), NH_3-N concentration should be greater than 10 mg/dL to maximize ruminal digestion,

Ammonia concentration (T_0) is in agreement with Patra et al. (2013). These authors, in a meta-analyzes study about lipid effects on ruminal parameters, observed N-NH_3 concentration of 12.9 mg/dL. Nonetheless, they observed high variability among analyzed studies, in a form in which the minimum concentration of N-NH_3 was 3.1mg/dL and the maximum was 34.5 mg/dL.

However, according to Van Soest (1994), the optimum N-NH_3 level cannot be a fixed value, because N-NH_3 utilization by microorganism and consequently microbial protein production are dependent to carbohydrates fermentation rate (energy availability). Thus, Ex situ methodology can evaluate N-NH3 concentration available in the rumen for microorganism, and through incubation it is possible to determine the N-NH3 amount after fermentation.

After fermentation (T_{30}), the N-NH_3 concentration was, on average, 23.2 mg/dL. Thereby, N-NH_3 balance was positive, on average 1.31 mg/dL/hour. Positive balance is an indicative that the amount of N-NH_3 in the rumen was enough for the microorganism growth and microbial protein production. High positive balance can be toxic for microorganisms and can cause damage to fermentation. Furthermore, it is indicative that N-NH_3 is not a limiting factor for the optimum fermentation or that there is a deficit energy content giving limitation for the fermentation.

According to Bergman et al. (1990), acetate is the mainly SCFA in the rumen, it can represent 75% of total SCFA. Propionate and butyrate are in low concentration when compared to acetate. Normally acetate, propionate and butyrate molar

concentration rate ranges from 75:15:10 to 40:40:20. In the present study, the acetate, propionate and butyrate concentrations (T_0) were 72:15:13 and 69:21:10 for the control and cottonseed treatments, respectively. Both treatments are in agreement with Coelho Silva and Leão (1979). According to these authors, SCFA has higher ruminal variation due to diet characteristics, thus the variation ranges can be 54% to 74% for the acetate, 16% to 27% for the propionate and 6% to 15% for the butyrate.

Dietary lipid supplementation may influence SCFA production, depending on the composition of the basal diet and the amount of lipid added (Benchaar et al. 2012; Chelikani et al. 2004; Shingfield et al. 2008). Data on literature shows variable results on the effect of dietary lipid supplementation on ruminal SCFA production. In the present study, propionate had similar production however, acetate and butyrate had lower production when cottonseed was included. Overall, ruminal fermentation was changed. Acetate and butyrate are produced from the same pathway, whereas propionate is produced from other pathways.

Acetate and butyrate have cellulose and hemicellulose as major precursors. These fibrous carbohydrates have a slower digestion rate in the rumen when compared to the non-fiber-carbohydrates. As a consequence, fibrous carbohydrates products are released slowly. Cottonseed inclusion increased cellulose and hemicellulose content in the cow's diets, resulting in slower acetate and butyrate production when compared to the control. Sullivan et al. (2005) reported that the acetate molar proportion and the acetate:propionate ratio decreased linearly with increased dietary FA from whole cottonseed.

On other hand, propionate has non-fibrous carbohydrate (NFC) as the major precursor. In spite of the fact that cottonseed diets had lower NCF, they had higher lipids. In the rumen, lipids cannot be fermented, but they are hydrolyzed. The product of lipid hydrolyzes is fatty acid and glycerol. Glycerol is quickly fermented by microorganisms, and then it is mainly converted in propionate. Thus, propionate had a similar production rate with different precursors.

Our experimental results indicate that enteric methane emissions decreased significantly (by approximately 42,3%) in the cottonseed diets when compared to the basal diet. The reduction in the intensity of CH_4 emissions (as g of CH_4/kg DM) by including cottonseed in diet was higher in our study than the studies of Martin et al. (2008) and Beauchemin et al. (2009b), where deceases were 27% and 18%,

respectively. This difference can be due high lipid content in the cottonseed diet compared with others authors.

Present results suggest that, for each percentage of lipids added in the cow diets, the result was a reduction of 8.3% in the methane emissions. This result was higher than that found by Patra et al. (2014). In a meta-analysis study, they concluded that, for each percentage of lipid added, the result was a reduction of 4,3% in the methane emissions. This difference is likely due the highest oil level used in the present experiment. Acccording to Beauchemin et al. (2008) in a meta-analysis study, they showed a linear relationship between the percentage of lipid added and the reduction in the CH_4 emissions.

The inhibitory effect of lipid on enteric methane emissions has been reported in the majority of studies, despite the extent of inhibition to be variable (Brask et al. 2013; Grainger and Beauchemin, 2011).

Several mechanisms have been recognized for the inhibitory effects of lipids on methane emissions. Lipids inhibit methanogenesis by reducing the metabolic activity and numbers of ruminal methanogens and protozoa.

Biohydrogenation of unsaturated fatty acids is an alternative hydrogen sink, and it decreases free hydrogen in the rumen (Beauchemin et al. 2009; Johnson and Johnson, 1995). Lipids are not fermented in the rumen, and thus, they do not produce a surplus of free hydrogen. Among the SCFA, acetate production releases the highest amount of free hydrogen in the rumen, hence by decreasing the acetate production, the free hydrogen concentration will be reduced. Consequently, methane production could be decline directly by reducing methanogens number and/or activity, and indirectly by production and/or concentration of less hydrogen, when higher cottonseed levels are included in the diets.

The pattern of the SCFA production and methane emissions during the day increased rapidly after feeding and then decreased slowly until the next feeding, SCFA and methane production peaked occurred immediately after post-feeding (Fig. 3), as previously demonstrated by Mao et al. (2010).

4.4.2. Energy partition

Overall, energy release in the rumen is related to SCFA and methane production, as well as ruminal solid mass. Cottonseed treatments had 32% higher ruminal solid mass compared to the control. Despite propionate production in the rumen, in which was expressed as ruminal mass, to be similar among treatments (Table 7), the highest ruminal solid mass in the cottonseed treatments induced a higher propionate release in the rumen. This was on order of 42%, when expressed as Mcal/ani/d, compared to the control. Acetate production was 33,8% lower (Table 7), when expressed as ruminal mass, for the cottonseed treatments when compared to the control. However, the higher ruminal solid mass for the cottonseed treatments cancels out this lower acetate production, and thereby the acetate release in the rumen, when expressed as Mcal/ani/d was similar among treatments.

In spite of higher ruminal solid mass for the cottonseed treatments, an expressive reduction in the butyrate production, on the order of 47% when expressed as ruminal mass (Table 7) was enough to further a decrease in the order of 32% in the energy release in the rumen, when expressed as Mcal/ani/d, compared to the control. A Similar situation was observed for the methane, in which a lower ruminal methane production on the order of 42% when expressed as ruminal mass (Table 7) was enough to further a decrease of ruminal energy release of 26%, when expressed as Mcal/ani/d for the cottonseed treatments compared to the control.

Changes in the energy release site occurred. In the rumen of cows fed cottonseed, less energy was released as methane and butyrate, when expressed as Mcal/ani/d, in percentage of GE or DE. Hence, more energy was released in the intestine, because the energy released in the feces was similar among treatments. Thus, energy release in the intestine, when expressed in Mcal/ani/d, was 57,3% higher for the cows fed cottonseed when compared to the control.

Methane production in cattle typically accounts for 5,5 to 6,5% of GEI (Johnson and Ward 1996); however, values of 2 to 12% (Johnson and Johnson 1995) have been reported for some diets. In the present study, our data are in agreement these authors, as CH_4 emissions averaged 6,27% of GEI to the cottonseed treatments and 9,72% of GEI to the control treatment. Because methane represents a loss of dietary energy, a significant reduction in these gas emissions was observed for the cows fed

cottonseed and indicated that these animals were more efficient in utilizing dietary energy than the control.

4.5. CONCLUSION

Cottonseed inclusion in the cattle diets can be considered as methane mitigation strategy. Changes in the ruminal products as lower acetate, butyrate and methane production are favorable to the use of cottonseed in cattle diets. The use of vitamin E is not advisable for cattle due to their inclusion did not result in improvements in ruminal fermentation, thus its addition would result in a financial raise without benefits.

REFERENCES

ANDRADE, J. G. Effects of feeding high oil corn to beef steers on housing characteristics and meat quality. **Journal of Animal Science**, v. 79, p. 582-588, 2010.

AOAC (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS). **Official methods of analysis**. 16 th ed. Virginia, AOAC, 1995.

BALDWIN, R. L.; ALLISON, M. J. Rumen metabolism. **Journal of Animal Science**, v. 57, p. 461-477, 1983.

BEAUCHEMIN,K. A.; KREUZER, M.; O'MARAC, F.; MCALISTER, T. A. Nutritional management for enteric methane abatement: a review. **Australian Journal of Experimental Agriculture**, v. 48, p. 21-27, 2008.

BEAUCHEMIN, K. A.; MCGINN, S. M.; PETIT, H. V. Methane abatement strategies for cattle: lipid supplementation of diets. **Journal Animal Science,** v. 87, p. 431-440, 2007.

BEAUCHEMIN, K. A.; MCGINN, S. M.; BENCHAAR, C.; HOLTSHAUSEN, L. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. **Journal of Dairy Science**. v. 92, p. 2118-27, 2009.

BEACHUMIN, K. A; MCALLISTER, T. A; MCGINN, S. M. Dietary mitigation of enteric methane from cattle**. CAB Review:** Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, n. 4:035, p. 1-18, 2009a.

BENCHAAR, C. G. A.; ROMERO-PEREZ, P. Y.; CHOUINARD, F.; HASSANAT, M.; EUGENE, H. V. Supplementation of increasing amounts of linseed oil to dairy cows fed total mixed rations: effects on digestion, ruminal fermentation characteristics, protozoal populations, and milk fatty acid composition. **Journal Dairy Science,** v. 95, p. 4578-4590, 2012.

BERGMAN, E. N. Energy contributions of volatile acids from gastrointestinal tract in various species. **Physilogical Reviews**, v. 70, p.567-581, 1990.

BLOOMBERG, B. D.; HILTON, G. G.; HANGER, K. .; RICHARDS, C. J.; MORGAN, J. B.; VANOVERBEKE, D. L. Effects of vitamin E on colour stability and

palatability of strip loin steaks from cattle fed distillers grains. **Journal of Animal Science,** v. 89, p. 3769-3782, 2011.

BRASK, M.; LUND, P.; WEISBJERG, M. R.; HELLWING, A. L .F.; POULSEN, M.; LARSEN, M. K.; HVELPLUND, T. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. **Journal of Dairy Science**, v. 96**,** p. 2356-2365, 2013.

BUDDLE, B. M.; DENIS, G. T.; ATTWOOD, E.; ALTERMANN, P. H.; JANSEN, R. S.; ROMINUS, C. S.; PINARES-PATINO, S.; MUETZEL, N. Strategies to reduce methane emissions from farmed ruminants grazing on pasture. **Veterinarian Journal**, v. 188, p.11-17, 2011.

CHELIKANI, P. K.; BELL, J. A.; KENNELY, J. J. Effect of feeding or abomasal infusion of canola oil in Holstein cows: 1. Nutrient digestion and milk composition. **Journal Dairy Research**, v. 71, p. 279-287, 2004.

CHILIBROSTE, P.; TAMMINGA, S.; BOER, H.; GIBB, M. J.; DIKKEN, G. Duration of regrowth of ryegrass (Lolium perene) effects on grazing behavior, intake, rúmen fill and fermentation of lacting cows. **Journal Dairy Science**, v. 83, p. 984-995, 2000.

CONCEIÇÃO, D.; JACQUES, R.; BENTO, F.; SIMONETTI, A.; PEDRO.; SELBACH, P.; CAMARGO, F. Redução de cromo hexavalente por bactérias isoladas de solos contaminados com cromo. **Ciência Rural**, v. 37, p. 1661-1667, 2007.

DINI, Y.; GERE, J.; BRIANO, C.; MANETTI, M.; JULIARENA, P.; PICASSO, V.; ASTIGARRAGA, L. Methane emission and milk production of dairy cows grazing pastures rich in legumes or rich in grasses in Uruguay**. Animals**, v. 2, p. 288-300, 2002.

DOREAU, M.; FERLAY, A. Effect of dietary lipids on the ruminal metabolism in the rumen: a review. **Livestock Production Science**, v. 43, p. 97-110, 1995.

ERWIN, E. S.; MARCO, G. J.; EMERY, E. M., Volatile fatty acids analyses of blood and rumen fluid by gas chromatography. **Journal of Dairy Science**,v. 4, p. 1768-1771, 1961.

FOLDAGER, J. **Protein requirement and non protein nitrogen for high producing cow in early lactation.** Thesis (PhD in Animal Science). Michigan State University, East Lasing, Michigan, USA, 1977.

GERBER, P. J.; STENFELD, H.; HENDERSON, B.; MOTTET, A.; OPIO, C.; DIJKMAN,J.; TEMPIO, G. **Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities**. Rome: Food and Agriculture Organization of the United Nations (FAO), 2013.

GRAINER, C.; BEAUCHEMIN, K. A. Can enteric methane emissions from ruminants be lowered without lowering their production? **Animal Feed Science and Technology,** v. 166**,** p. 308-320, 2011.

HUERTA-LIENDENZ, N. O. Grow carcass traits, and fatty acid profiles of adipose tissues from steers fed whole cottonseed. **Journal Animal Science**, v. 69, p. 3665-3672, 1991.

HOU, J.; WANG, F.; WANG, Y.; LIU, F. Effects of vitamin E on the concentration of conjugated linoleic acids and accumulation of intermediates of ruminal biohydrogenation in vitro. **Small Ruminant Research**, v. 111, p. 63-70, 2013.

JOHSON, K. A.; JOHNSON, D.E. Methane emissions from cattle. **Journal of Animal Science,** v. 73, p. 2483-2492, 1995.

JUAREZ, M.; DUGAN, M.E.R.; ALDAI, N.; AALHUS, J.L.; BASARAB, J.A.; BARON, V.S. Dietary vitamin E inhibits the trans 10–18:1 shift in beef backfat. **Canadian Journal of Animal Science** v.90, p.9–12, 2010.

KAMINSKI, M.; KARTANOWICZ, R.; JASTRZEBSKI, D.; KAMINSKI, M.M. Determination of carbon monoxide, methane and carbon dioxide in refinery hydrogen gases and air by gas chromatography. **Journal of Chromatography**, v. 989, p. 277-283, 2003.

KULASEK, G. A. A micromethod for determination of urea in plasma, whole blood cells using urease and phenol reagent. **Polskie Archiwum Weteraryjne**, v. 15, p. 801-810, 1972.

LENG, R. A. Factors affecting the utilization of "poor-quality" forages by ruminants particularly under tropical conditions. **Nutrition Reserve Review**, v. 3, p. 277-300, 1990.

MAO, H. L.; WANG, J. K.; ZHOU, Y. Y.; LIU, J. X. Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. **Livestock Science**, v. 129, p.56-62, 2010.

MARTIN, C.; ROUEL, J.; JOUANY, J. P.; DOREAU, M.; CHILLIARD, Y. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. **Journal of Animal Science**, v. 86**,** p. 2642–2650, 2008.

NARDONE, A.; RONCHI, B.; LACETERA, N.; RANIERI, M. S.; BERNABUCCI, U. Effects of climate changes on animal production and sustainability of livestock systems. **Livestock Science**, v. 130, p. 57-69, 2010.

PATRA, A. K. A meta-analysis of the effect of dietary fat on enteric methane production, digestibility and rumen fermentation in sheep, and a comparison of these responses between cattle and sheep. **Livestock Science**, v. 162, p. 97-103, 2014.

PENNER, G. B.; BEAUCHEMIN, K. A.; MUTSVANGWA, T. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. **Journal of Dairy Science,** v. 89, p. 2132-2140, 2006.

PESCE, D.M.C. **Efeito da dieta contendo caroço de algodão no desempenho, característica quantitativa de carcaça e qualitativa da carne de novilhos Nelore confinados**. Pirassununga. 155f. Tese (doutorado em Zootecnia). Curso de Pós-Graduação em Zootecnia. Universidade de São Paulo, São Paulo, 2008.

RODRIGUES, P. H. M.; PINEDO, L. A.; ROMERO, L. A. S.; PERNA JUNIOR, F.; MARTINS, M. F.; CASTRO, A. L.; GDODOY, G. L. A.; MARINO, C. T. Descrição da metodologia ex situ de estudo da fermentação ruminal (micro-rúmen) com vistas à mensuração da produção de metano. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 49., 2012, Brasília, DF. **Anais…** Brasília, SBZ, 2012.

SATTER, L.D.; SLYSTER, L.L. Effect of ammonia concentration on rumen microbial protein production in vitro**. Bristish Journal of Nutrition**, 32, 199-208, 1974.

SHINGFIELD, K. J.; AHVENJARVI, S.; TOIVONEN, V.; VANHATALO, A.; HUHTANEN, P.; GRIINARI, J. M. Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. **Brazilian Journal of Nutrition,** v. 32, p. 199-208, 1974.

SULLIVAN, H.M.; BERNARD, J.K.; AMOS, H.E. Ruminal fermentation and amino acid flow in Holstein steers fed whole cottonseed with elevated concentrations of free fatty acids in the oil. **Journal of Dairy Science**, v. 88, p.690-697, 2005.

ZAKRYS, PI.; HOGAN, S. A.; O'SULLIVAN, M. G.; ALLEN, P.; KERRY, J. P. Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. **Meat Science,** v. 79, p. 64-655, 2008.

WEI, C.; LIN, S.X.; WU, J. L.; ZHAO, G. Y.; ZHANG, T. T.; ZHENG, W.S. Effects of supplementing vitamin E on in vitro rumen gas production, volatile fatty acid production, dry matter disappearance rate, and utilizable crude protein. **Czech Journal of Animal Science**, v.60, p. 335-341, 2015.

5. DIET EFFECTS ON BIOCHEMICAL METHANE POTENTIAL, NUTRIENTS REMOVAL AND GROSS ENERGY RECOVERY IN LABORATORY BATH DIGESTION OF CATTLE FECES

Abstract: When feces are stored under anaerobic conditions they are a source of emissions of greenhouse gas, mainly methane. Different diets can changes feces characteristics and hence they could change the methane emissions. Anaerobic digestion of cow feces was performed to evaluate the effects of different diets on ingestate and digestate characteristics, in addition the methane yield. Cows were fed with different diets. Feces samples were loaded into batch anaerobic digesters. Treatments for the digesters included: 1) Control, feces from cows fed control diet; 2) CS, feces from cows fed CS diet and 3) CSVitE, feces from cows fed CSVitE diet. Analyses of nutrients were conducted for ingestate and digestate samples. Biogas production was measured and methane concentration was analyzed. Methane concentration in biogas was higher for cottonseed treatments, 80% vs. 74%, compared to the control. Lower concentration of phosphate in the cottonseed digestate was observed, 8,1 vs. 14,5g/L, compared to the control. Removal efficiency of OM and CP was decreased in 7% and 13% respectively, for cottonseed treatments compared to the control. Gross energy partition in the anaerobic digestion was similar among treatments. On mean, 28%±1,23 gross energy fed was release as methane, 47%±1,59 was release in the digestate and 25%±2,07 was release as others gases and heat. High cottonseed level in the cattle diet changed the ingestate characteristics, however both cottonseed and vitamin E, when added in cattle diets did not changed methane yield.

Keywords: Anaerobic digestion, cottonseed, methane yield, vitamin E.

5.1. INTRODUCTION

Approaches to mitigate enteric methane in ruminants by manipulating diet type and quality, including lipid supplementation, have been adopted and some results indicate a positive effect (Møller et al. 2016). However, the inclusion of lipid can have use limited by possibility of unsaturated fatty acids in suffer lipid peroxidation (Zakrys et al. 2008). Dietary lipids such as supplemental oil or oilseeds, if not stabilized, can be significant contributors to the load of free radicals in the animal (Andrews et al. 2006).

In addition, a small amount of oxygen may go to the rumen with saliva, feeds, drinking water and diffusion from blood into rumen, it has as consequences a release free radicals in the rumen (Wey et al. 2015).

A main way to reduce or prevent lipid peroxidation is through the use of antioxidants, and generally, through substances or conditions with preventive oxidation properties. In recent years, supplemental vitamin E in the diet of ruminants has been studied for its potential role in give stability to lipid deposits and improving the resistance to oxidation (Bloomberg et al. 2011). Therefore, supplementing vitamin E to relieve oxidation effects from free radicals could be beneficial to rumen fermentation with improvements in the nutrient digestibility and consequences in thier excretions.

However, effects of changing diets in terms of biogas potential and potential methane (CH4) losses from feces has not been studied thoroughly (Møller et al. 2014). Although different feeding strategies can reduce enteric CH_4 emission from ruminants or improve the ruminal conditions, the subsequent effects on feces biogas production and methane yield remains unclear. In fact, most of these strategies are based on variations in the digestibility of nutrients. Therefore, by using these strategies, alterations on biodegradable organic content in the feces can be expected and consequently on methane yield (Møller et al 2014).

Biochemical methane potential (BMP) test has been conducted in laboratory batch scale, measuring the maximum amount of biogas or bio-methane produced per gram of volatile solids (VS) contained in the substrates of the anaerobic digestion process (Sommer et al. 2004). Thorough investigation of methane potential yield is a pre-requisite to better predict CH_4 emission by anaerobic digestion or during feces storage in anaerobic conditions (Bloomberg et al. 2011).

The objectives of this study were to evaluate the effects of established dietary strategies to mitigate enteric CH4 emission or prevent lipid peroxidation on ingestate and digestate characteristics, as well as in the methane yield and in the potential of digester to recovery from feces energy to methane.

5.2. MATERIAL AND METHODS

5.2.1. Study location and compliance requirement

This study was performed at University of Sao Paulo, Pirassununga, Brazil. The experiment was approved and complied with the guidelines set out by the "Ethics Committee in the Use of Animals of the University of São Paulo", under application number nº 009/2013, in regards to animal experimentation and care of animals used for scientific purposes.

5.2.2. Animal feeding, housing and feces collected

Six Holstein dairy cows not pregnant and non-lactating with an average body weight of 876 kg (±16.1) were arranged in individual pens with free access to water and sand bedded. Cows were fed ad libitum twice daily (08h00 and 16h00). The vitamin E amount was weighted daily to offer 500 IU per animal per day and the level was established according to Hansen et al. (2004). Vitamin source was Lutavit E 50 BASF, with 50% alpha tocopheryl acetate. Fatty acids and cottonseed level in the diet was established according to previous study (Montgomery et al. 2005). The ingredient proportion and chemical composition of the diets are given in Table 1.

For feeding and feces collection, cows were arranged in 3x3 Latin square designs with three experimental periods, each one lasted 21 days. The first 10 days of each test period were used for diet adaptation. Feces collection was conducted during the next 11^{th} to 17^{th} days. Feces were collected, via rectal, twice daily at 08h00 and 16h00 after feeding.

5.2.3. Substrates, experimental design and treatments

Representative pools were collected from feces of each animal, fed with different diets, in each period. Feces pools were then diluted in water using a 7:1 ratio, according to methods in the literature (Huerta-Leidenz et al. 1991). Eighteen laboratory scale digesters made from pipes with 2 liters of capacity (Fig. 3) were loaded with different substrates.

Anaerobic digestion test was carried out under mesophilic conditions. Treatments were determined based on feces from cows of different diets. Digesters were arranged in a completely randomized design with 3 treatments and 6 repetitions. Digesters were started up using the substrates, no inoculum were used. Treatments and respective substrates characterization are showed in table 9.

Table 9. Characteristics of substrates used in batch digester test

	Treatment				Probability	
	Control	CS	CSVitE	SEM	C 1	C 2
TS, (g/kg)	18,7	22,5	22,5	0,54	0,0003	NS
VS, (g/kg)	16,7	20,4	21,0	0,51	0,0001	NS
[1]OM, (g/kg TS)	806	819	824	5.10	NS	NS
CP, (g/kg TS)	127	133	133	2,52	NS	NS
EE, (g/kg TS)	15,6	16,5	14,4	0,61	NS	NS
NDF, (g/kg TS)	399	453	472	16,7	0,0626	NS
ADF, (g/kg TS)	309	379	412	17,1	0,0147	NS
Lignin, (g/kg TS)	99,3	187	137	14,9	0,0357	NS
GE, (Mcal/kg TS)	4,06	4,28	4,27	0,50	0,0609	NS

TS: total solid; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; GE: gross energy; SEM: standard error of mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10)
[1] OM: DM-minerals.
Font: Nogueira, 2017.

5.2.4. Biogas and methane measurement

Frequency of biogas measurement was conducted following gasometer capacity. Biogas volume was calculated using gasometer vertical displacement (Fig. 4), which was measured in centimeters. Biogas volume was determined by the

displacement and gasometers internal cross-sectional area, and corrected to 1 atm and 20ºC according to methods published (Lucas Junior, 1994).

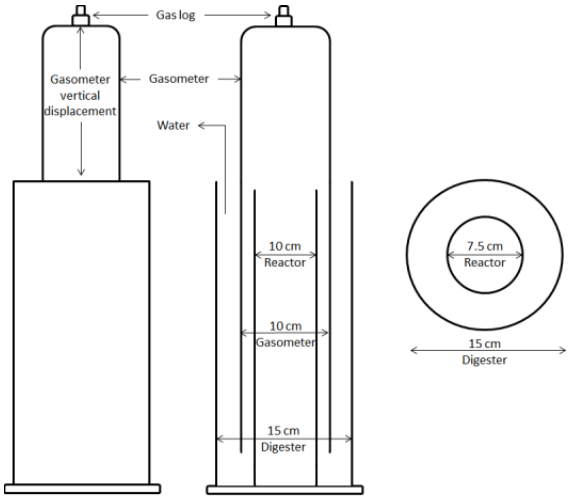
Biogas samples were conducted every time that biogas volume was measured. Samples were collected using a syringe connected to the gas log on top of the gasometer (Fig. 4). Sample was first used to flush the glass bottles, the flushing was done twice. After that, 50 mL of biogas was injected into the glass bottles for biogas composition analysis.

Methane composition was analyzed for the entire biogas sample. For each sample, 2 ml of biogas was collected from the glass bottles with *Gas-Tight* syringe and injected into a Thermo Scientific Trace 1310 chromatograph, equipped with a flame ionization detector at 280°C, with 3,5 m Porapak N (Supelco) column. The chromatograph was calibrated with 3.1% methane, 3.1% carbon dioxide, and 0,49% of nitrous oxide, diluted in atmosphere air. A gas mixture with 50% CH_4 and 50% CO_2 was used as the reference gas. The carrier gas was helium and flow rate was 30 mL $minute^{-1}$. Methane volume was calculated by multiplication of biogas volume and methane concentration.

Specific methane yield (per gram of VS fed or destroyed) was calculated by dividing the total methane production (L) by amount of volatile solids fed (before AD), or destroyed (difference between VS fed and eliminated). For the methane yield

The test was terminated when biogas production ceased.

Figure 4. Anaerobic digester shown in front, side, and top views.



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5.2.5. Nutrients removal

Ingestate and digestate were weighed and multiplied by DM content, in percentage, to calculate the DM content in grams. Nutrients of ingestate and digestate, when express as g, were calculated by multiplication between digestate or ingestate, express as gram of DM and ingestate or digestate nutrients, express in percentage, divided by 100 according to the follow equation:

$$Nutrients\ (g) = \frac{nutrient\ fed\ or\ eliminated\ (\%) \times DM\ (g)}{100}$$

Nutrients removals, when express as percentage, were calculated using digestate and ingestate nutrient contents, express as grans per kilogram of DM, according to the equation:

$$\text{Nutrients removal}(\%) = \frac{\text{nutrient fed - nutrient eliminated}}{\text{nutrient fed}} \times 100$$

5.2.6. Gross energy partition

Methane gross energy release, when express as Mcal, was calculated using total methane production, in liters, and information about the methane molecule:

Molar volume: 26,22 mol/L
Molar mass: 16,04 g/mol
Heat power: 13,16 kcal/g

Other gases and heat release, when express as Mcal, was determined by gross energy fed minus energy release as methane minus gross energy eliminated in the

digestate. The percentage of gross energy release when express as percentage of gross energy fed was calculated by division between gross energy of methane or others gas and heat or digestate and gross energy fed, multiplied by 100.

5.2.7. Laboratory analysis

Individual feed samples and substrates, pre and post anaerobic digestion, were collected and composited in representative samples on an equal-weight basis. Samples were dried at 60°C (forced-air oven) for 48 h and ground to pass a 1-mm Wiley mill screen and analyzed. DM concentration was determined following methods in the literature (AOAC, 1995) in the forced-air-oven at 105°C for 2 h followed by cold weighing (method 930.15). Nitrogen content was determined by the micro Kjeldahl method, and it was multiplied by 6.25 to determine CP (AOAC, 1995). Ether extract was determined according to Association of Official Analytical Chemists (AOAC, 1995), using light petroleum ether in the Soxhlet apparatus (method 920.39). Neutral detergent fiber, ADF, and lignin was determined according to methods in the literature (Van Soest et al. 1991), using Ankom® Filter Bag technique and heat stable α−amylase added (method 973.18).

Total solid and volatiles solids were analyzed using method 1684 (EPA, 2001). The procedures included preparing evaporating dishes and heating clean evaporating dishes at 550ºC for 1 hour in a muffle furnace. Samples were first homogenized, and about 30 g of sample aliquot was then placed on prepared evaporating dishes, and weighed to the nearest precision 0,01g. The samples were dried at 105ºC in an oven for 12 hours, then cooled during 1 hour to balance temperature in a desiccator containing fresh desiccant and weighted. For volatile solids analysis, the evaporating dishes containing the dried residues were then put into muffle furnace and heated to 550º C and for at least 4 hours. The residues were cooled in a desiccator to balance the temperature for 2 hours and weighed.

5.2.8. Statistical analysis

The experimental design for the study was a completely randomized design with 18 experimental units (digesters) for 3 treatments (control, CS, and CSVitE) and 6 repetitions. Data were tested for residual normality using the Shapiro-Wilk test. Data statistical analyses were performed with the Statistical Analysis System software (Version 9.3 SAS Institute Inc., Cary, NC, USA) using Mixed model. Analysis included descriptive statistics, where mean values and standard errors of the mean were calculated. The model included the fixed effect of treatment. The variables were analyzed using the following model:

$$Y_{ijkl} = \mu + T_i + e_{ijk},$$

Where:

Y_{ijkl} = the dependent response variable

μ = the overall mean

T_i = treatment effect

e_{ij} = the residual error term.

Non-significant (NS) was considered when P value was higher than 10%.

Contrast statements were used to evaluate differences between treatments: 1) Control vs. CS and CSVitE (C1) and 2) CS vs. CSVitE (C2). Statistical significance was declared at $P \leq 0,05$.

5.3. RESULTS

5.3.1. Total solid and volatile solid

Differences in substrate composition were observed (Table 9). Cottonseed treatments had higher concentration of TS (22,5 g/kg vs. 18,7 g/kg) and VS (20,g/kg%

vs. 16,7 g/kg), as well as higher ADF (395 vs. 309 g/kg TS) and lignin (162 vs. 99,3 g/kg TS) when compared to the control.

As shown in Table 10, significant differences were observed cottonseed treatments had higher elimination of TS (11,4 g/kg vs. 10,0 g/kg) and VS (9,4 g/kg vs. 7,9 g/kg) when compared to the control. No differences were observed for the TS destroyed and VS destroyed. On mean, treatments had 49,6% and 53,7% for the TS destroyed and VS destroyed, respectively.

Table 10.Total and volatile solids destroyed in batch digester loaded with feces from cows fed different diet

	Treatment				Probability	
	Control	CS	CSVitE	SEM	C 1	C 2
$TS_{eliminated}$,(g/kg)	10,0	10,9	11,9	0,21	0,0202	NS
$VS_{eliminated}$, (g/kg)	7,91	9,00	9,82	0,21	0,0106	NS
$TS_{destroyed}$, (%)	47,7	52,6	48,6	1,34	NS	NS
$VS_{destroyed}$, (%)	52,5	55,5	53,1	1,43	NS	NS

TS: total solid; VS: volatile solid; SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
Font: Nogueira, 2017.

5.3.2. Biogas and methane yield

No effect was observed for the total biogas, total methane and methane yield, averaging 8,0 L of biogas, 6,3 L of methane, 0,024 L CH_4/g feces-fed, 0,16 L CH_4/g VS-fed and 0,03 L CH_4/g VS-destroyed. Methane concentration in the biogas was higher for cottonseed treatments (79,8% vs. 74,0%) when compared to the control (Table 11).

Table 11. Biogas and methane yield and methane concentration of batch digesters loaded with feces from cows fed different diets

	Treatment				Probability	
	Control	CS	CSVitE	SEM	C 1	C 2
$Biogas_{total}$, (L)	7,593	8,256	8,373	0,403	NS	NS
$CH_{4Concentration}$, (%)	74,39	79,69	80,05	0,842	0,0006	NS
CH_{4total}, (L)	5,637	6,573	6,701	0,320	NS	NS
CH_4/ $feces_{fed}$, (L/g)	0,022	0,026	0,026	0,001	NS	NS
$CH_4/VS._{fed}$, (L/g)	0,168	0,166	0,159	0,007	NS	NS
$CH_4/VS._{des}$, (L/g)	0,320	0,290	0,300	0,014	NS	NS

TS: total solid; VS: volatile solid; des: destroyed; SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
Font: Nogueira, 2017.

5.3.3. Fertilizer value

As shown in the table 12, lower P_2O_5 concentrations were observed for the cottonseed treatments (8,1 vs. 14,5 g/L) when compared to the control. No difference was observed for the Carbon, nitrogen and K_2O contents; on mean the digestate had 376 g/L of carbon, 21,7 g/L of nitrogen, and 8,1 g/L K_2O.

Table 12. Minerals content of digestate of batch digesters loaded with feces from cows fed different diets

	Treatment				Probability	
	Control	CS	CSVitE	SEM	C 1	C 2
C, (g/L)	380	376	373	16,1	NS	NS
N, (g/L)	19,7	22,4	23,0	7,17	0,0519	NS
P_2O_5, (g/L)	14,5	8,80	7,50	1,36	0,0265	NS
K_2O, (g/L)	8,00	8,40	7,90	1,86	NS	NS

SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
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5.3.4. Nutrients removal

Table 13 shows ingestate and digestate nutrients express as gram. As can be

observed cottonseed treatments had higher DM (45 vs. 37.4 g), NDF (20.8 vs.14.9 g), ADF (17.8 vs. 11.6 g) and lignin (5.38 vs. 3.77 g) fed when compared to the control.

For the digestate, cottonseed treatments had higher DM (22.1 vs. 19.5 g), OM (16.1 vs. 13.4 g), CP (3.1 vs. 2.4 g), NDF (12.1 vs. 8.7 g), ADF (9.9 vs.6.4 g) and lignin (3.5 vs. 1.87 g) eliminated when compared to the control (Table 13).

Cottonseed inclusion decreased removal efficiency for the OM (51 vs. 58.7 %) and CP (41.3 vs. 54.4%) when compared to the control. No effect was observed for the others nutrients removal, and on mean was 64,3 % for the DM, 53,1% for the NDF, 55,4% for the ADF, 57,4% for the lignin and 47,6% for the EE (Table 13).

Table 13. Ingestate and digestate nutrients content, and removal efficiency of batch digesters loaded with feces from cows fed different diets

	Treatment				Probability	
	Control	CS	CSVitE	SEM	C 1	C 2
Nutrients fed						
DM, (g)	37,4	45,1	45,0	1,23	0,0003	NS
[1]OM, (g)	32,6	33,1	33,1	0,21	NS	NS
CP, (g)	5,15	5,39	5,41	0,09	NS	NS
NDF, (g)	14,9	20,4	21,3	0,97	0,0019	NS
ADF, (g)	11,6	16,9	18,7	1,06	0,0027	NS
Lignin, (g)	3,77	5,48	5,28	0,38	0,0456	NS
EE¸ (g)	0,58	0,74	0,64	0,002	NS	NS
Nutrients eliminate						
DM, (g)	19,5	21,2	23,1	0,57	0,0184	NS
[1]OM, (g)	13,4	15,5	16,7	0,54	0,0073	NS
CP, (g)	2,38	2,99	3,31	0,13	0,0048	NS
NDF, (g)	8,75	11,7	12,6	0,51	0,0003	NS
ADF, (g)	6,40	9,21	10,6	0,46	0,0001	NS
Lignin, (g)	1,87	3,32	3,73	0,27	0,0003	NS
EE¸ (g)	0,41	0,45	0,56	0,03	NS	NS
Removal efficiency						
DM, (%)	47,7	52,6	48,6	1,34	NS	NS
[1]OM, (%)	58,7	53,2	49,8	1,37	0,0115	NS
CP, (%)	54,4	44,6	38,0	2,50	0,0078	NS
NDF, (%)	39,6	41,1	40,6	2,61	NS	NS
ADF, (%)	44,3	41,7	41,6	2,89	NS	NS
Lignin, (%)	43,0	39,2	26,5	6,04	NS	NS
EE, (%)	41,6	38,0	27,6	3,19	NS	NS

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
[1] OM: DM-minerals.
Font: Nogueira, 2017.

5.3.5. Gross energy partition

Cottonseed treatments had higher gross energy fed (0.19 vs. 0.15 Mcal) and gross energy release in the digestate (0.088 vs. 0.074 Mcal) when compared to the control. No differences were observed for the gross energy release when express as percentage of gross energy fed. On mean, 28% of gross energy fed was released as methane, 25.0% as others gas and heat and 47% as released in the digestate (Table 14).

Table 14. Gross energy release and eliminated by batch digesters loaded with feces from cows fed different diets

	Treatment				Probability	
	Control	CS	CSVitE	SEM	C 1	C 2
Gross energy fed, kcal	0,15	0,19	0,19	0,054	0,0001	NS
Methane release						
Mcal	0,045	0,052	0,053	0,002	NS	NS
Percentage of GE fed, %	28,9	27,1	27,9	1,23	NS	NS
Digestate release						
Mcal	0,074	0,085	0,092	0,002	0,0067	NS
Percentage of GE fed, %	48,1	44,3	48,2	1,59	NS	NS
Others gas and heat release						
Mcal	0,036	0,055	0,046	0,004	0,0933	NS
Percentage of GE fed, %	22,9	28,4	23,8	2,07	NS	NS

GE: gross energy; SEM: standard error of the mean; C1: contrast 1 (CS and CSVitE vs. control); C2: contrast 2 (CS vs. CSVitE); NS: non-significant (p> 0,10).
Font: Nogueira, 2017.

5.4. DISCUSSION

5.4.1. Substrates characteristics, total solids and volatile solids

Present data is in agreement with previous studies (Canh et al. 1997 and Jarret et al. 2011), in which modifications in diet composition affected the composition of the effluent, and in this case, especially the fibrous content. Fibrous content are characterized by low digestibility in ruminants and the higher fibrous contents were

provided by CS and CSVitE diet is the explanation for the higher TS, VS, NDF, ADF and lignin in the substrates. On the other hand, although there were higher EE contents in the CS and VitE diet, the high digestibility this compound by ruminants canceled out the differences between the EE in these substrates.

Volatile solids destroyed are commonly used to measure the performance of AD processes and it is a direct indicator of the metabolic activity of the microorganism community. For industrial scale digesters under mesophilic conditions, a 40% VS destroyed is an acceptable value (Rimkus et al. 1982). Destruction of VS in cattle manure in the AD process is typically in the range of 30–45% (Davidsson et al. 2008). In this study, regardless of the treatment, TS and VS destructions were over 45%, and were indicative of a sludge stabilization process and a good reactor performance in the batch digesters. Wilkie, (2005) assumed that 50% of VS are degradable, which is in agreement with the study, in which VS destruction was in average 53.7%.

5.4.2. Biogas and methane yield

Specific methane productivity, measured in terms of VS destroyed, typically corresponds to the theoretical methane yield (IPCC, 2006). It indicates degradation completeness of organic components of the feces. The methane productivity, in terms of VS_{fed}, is referred to as the ultimate methane yield (IPCC, 2006). Ultimate methane yield will always be lower than the theoretical yield because a fraction of the substrate is used to synthesize bacterial mass, thus a fraction of the organic material will be lost in the effluent, and lignin-containing compounds will only be degraded to a limited degree (Franco et al 2007). The results of this study are in agreement these authors, in which the ultimate methane yield was 54% of the theoretical methane yield. In addition, this data agreed with the VS reduction, where was 53,7%.

A theoretical methane yield for cattle manure was reported as 0,46 L CH_4/g VS-destroyed (Angelidaki et al.2000). It was also reported that emission factors reached as high as 0,4 L CH_4 per gram of volatile solid for manure which was held in management systems such as anaerobic lagoons (Møller et al. 2004). Present data are lower than the reports in literature, on average, 0,30 L CH_4/g VS-destroyed. This is due to high fibrous content within the digesters.

Volumetric methane yield of 0,13-0,16 L CH_4/g VS-fed were found in Pratt's et al. (2016) study. Other studies have (IPCC, 2006) reported 0,17 L CH_4/g VS-fed and variation of 0,07-0,28 L CH_4/g VS-fed (Gopalan et al. 2013). Present data are in agreement with those reported; the average volumetric methane yield was 0,16 L CH_4/g VS-fed. However, it has been observed that both, ultimate methane and theoretical methane yields produced by manure of different origins, can be highly variable and it is affected by various factors, including: species, breed and growth stage of the animals, feed, amount and type of bedding material and degradation processes during pre-storage (Angelidaki et al. 2000).

In spite of the fact that feces characteristics were affected by cattle diets, no differences were observed for the biogas production and methane yield in this study. Similar results were found by other authors who study the effect of different diets on CH_4 emission from dairy cows and their slurry. Hindrichsen et al. (2005) compared six different concentrate diets, such as oat hull, soybean hull, apple pulp, Jerusalem artichoke, molasses and wheat. Treatments effects were not significant. The above results were supported by the works of Yohanes (2010). Mathot et al. (2012) noticed that diet had no significant effect ($P>0,05$).

In the present study it was assumed that we could have minimized our results when compared to the others study due to present results were from feces whereas other study manure are considered. It is necessary to consider manure as feces together with urine, feed losses, bedding ending up and water spillage. Urine is hydrolyzed to inorganic nitrogen already during housing of the animal, and there will be no energy available in the urine fraction for biogas production. However, feed losses and bedding ending up in the manure will have an influence on the biogas potential. Water spillage to the manure system will also have a large influence on the volumetric biogas potential. (Mooler et al. 2014).

5.4.3. Fertilizer value

Studies using feces of high moisture content (in slurry form) for anaerobic digestion have been producing fertilizer products containing nitrogen (N), phosphorus (P) and potassium (K) of highly variable concentrations, which affect their use as

fertilizers in agriculture and also the amount of fertilizers applied onto agricultural lands. Digestate was reported to have higher N, P, and K contents than other nutrients (Tambone et al. 2010). In this study, the N content was significant in all digestate. The NPK ratio was found to be 100:73:40, 100:39:37, and 100:32:34 for the control, CS and CSVitE treatments, respectively. A difference was reported for the P_2O_5 concentration (C1=0,0265). These confirm that variation of nutrient contents does not only occur between digestate of different biogas plants, but also those found within one single biogas plant over time.

Concentrations of N and P_2O_5 of the digestate in the current study are similar to the range found reported (Chadwick et al. 2007; Smith et al. 2007). According to Chambers et al. (2014), N concentration was 26 g/L and P_2O_5 was 12 g/L. However, the reported K_2O concentrations of 32 g/L, was higher than the one measured in this study. As a whole, the evaluation of fluxes of important elements such as the macronutrients (N, P, and K), especially for agricultural biogas facilities is necessary for the utilization of digestate as fertilizers (Massé et al 1997).

5.4.4. Nutrients removal

The microorganisms from the control degraded more protein and organic matter than the microorganisms from CS and CSVitE (Table 3). These results suggest that microorganisms in the control had to hydrolyze higher quantities crude protein and organic matter to obtain nutrients. In contrast, microorganisms in CS and CSVitE had to hydrolyze higher quantities from NDF provided by the cottonseed inclusion to obtain nutrients. As consequence, both treatments had similar VS destruction, DM removal efficiency, in addition to methane yield.

Although the CS and CSVitE have higher DM available for the anaerobic digestion, the nutrients were fibrous content, NDF, ADF and lignin. Thereby, difference in the fiber amounts was not enough to improve the fibers removal efficiency and methane yield. These results suggest maximum removal efficiency for the fiber content was attained regardless of the availability of the microorganism.

Astals et al. (2012) studied nutrients removal efficiency in semi-continuous digesters loaded with pig manure and pig manure in co-digestion with glycerol. The

authors found removal efficiency for the crude protein 55,5%±19,3 and 25,2±10,8%, for the lipid 69,9%±5,2 and 34,9%±14,1 and for the fiber content 30,3%±21,1 and 11,0%±7,8 for the single digestion and co-digestion, respectively. Present data showed that it had high disparity compared to the above data; additionally, both data had high standard error of the mean. Therefore more studies with a safe number of replications to determine the nutrients removal efficiency are necessary.

5.4.5. Gross energy partition

Feces from cattle contain energy that can be recovered by various processes. The anaerobic digestion of feces to generate biogas, which is rich in methane, is an interesting way to recovery feces gross energy. In the present study the BMP test had a capacity to recovery 27,9% gross energy as methane, and it can be used for generation electricity or heat power. It is in agreement with McKendry (2002), who observed that the biomass is converted by bacteria in an anaerobic environment and produced a gas with an energy content of about 20–40% of the heating value of the feedstock

5.5. CONCLUSION

Cottonseed inclusion in the cattle diets changed ingestate characteristics to load the digesters, as well the digestate. However for both, cottonseed and vitamin E inclusion, no effect was observed in relation to methane yield. Anaerobic digestion in digester has a potential to recovery 28% gross energy lost in the feces into methane.

REFERENCES

ANGELIDAKI, B. K.; AHRING, B. K. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. **Water Science and Technology**, v. 41, n. 3, p. 189-194, 2000.

AOAC (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS). **Official methods of analysis**. 16 th ed. Virginia: AOAC, 1995

BLOOBERG, B. D.; HILTON, G. G.; HANGER, K. G.; RICHARDS, C. J.; MORGAN, J. B.; VANOVERBEKE, D. L Effects of vitamin E on color stability and palatability of strip loin steaks from cattle fed distillers grains. **Journal of Animal Science**, v. 89, p. 3769-3782, 2011.

CAETANO, L. **Preposition of the modficated system for the biogas quantification.** 75f. Master Science dissertation, Dept. Energy in Agriculture, Sao Paulo State University, Botucatu, Brazil, 1985.

CHADWICK, D. R. Digestate as a fertiliser and environmental concerns.In: ANAEROBIC DIGESTION STAKEHOLDER WORKSHOP, 2007. **Proceedings…**Exeter University, 2007.

CHAMBERS, B. J.; TAYLOR, M. J.; WITHERS, P.J.A. **Biosolids good practice guidance**. London: United Kingdom Water Industry Research, 2014. Report number SL11A/301.

CANTH, T. T.; VERSTEGEN, A.; ARMINK, A. J.; SCHARMA, J. W. Influence of dietary factors on nitrogen partitioning and composition of urine and feces of fattening pigs. **Journal of Animal Science**, v. 75, n. 3, p. 700-706, 1997.

DAVIDSSON, A.; LOVSTEDT, C.; JANSEN, .J. L.; GRUVBERGER, C.; ASPEGREN, H. Co-digestion of grease trap sludge and sewage sludge. **Waste Manage**, v. 28, n. 6, p. 986-992, 2008.

FRANCO, A.; MOSQUERA-CORRAL, A.; CAMPOS, J.L.; ROCA, E. Learning to operate anaerobic bioreactors. In: A. Méndez-Vilas (Ed.). **Communicating current research and educational topics and trends in applied microbiology.** Badajoz: Formatex, 2007. v. 2, p. 618-627.

GOPALAN, P.; JENSEN, P. D.; BATSTONE, D. J. Biochemical methane potential of beef feedlot manure: impact of manure age and storage. **Journal of Environmental Quality**, v. 42, p. 205-1212, 2013.

HANSEN, T. L.; SCHMIDT, J. E.; ANGELIDAKI, I.; MARCA, E.; JANSEN, J. C.; MOSBBAEK, H.; CHIRSTENSEN, T. H. Measurement of methane potentials of solid organic waste. **Waste Manage,** v. 4, p. 393-400, 2004.

HUERTA-LEIDENZ, N. O.; CROSS, H. R.; LUNTL, D. K.; PELTON, L. S.; SAWELL, J. W.; SMITH, B. Growth, carcass traits, and fatty acid profiles of adipose tissues from steers fed whole cottonseed. **Journal of Animal Science**, v. 69, p. 3665-3672, 1991.

IPCC (INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE).Emissions from livestock and manure management. In: **IPCC GUIDELINES FOR NATIONAL GREENHOUSE GAS INVENTORIES**. Hayama: Institute for Global Environmental Strategies, 2006. v. 4 Agriculture, forestry and land use.

LUCAS JUNIOR, J. **Considerations about swine manure use as substrates for three anaerobic digestion systems**. Ph.D. Dissertation. Dept. Agricultural Engineering, Sao Paulo State University, Jaboticabal, Brazil, 1994.

JARRET, G.; MARTINEZ, J.; DOUMAND, J.Y. Effect of biofuel co-products in pig diets on the excretory patterns of N and C and on the subsequent ammonia and methane emissions from pig effluent. **Animal**, v. 5, n. 4, p. 622-631, 2011.

MASSÉ, D. I.; DROSTE, R. L.; KENNEDY, K. J.; PATNI, N. K.; MUNROE, J. A. Potential for the psychrophilic anaerobic treatment of swine manure slurry in sequencing batch reactors. **Canadian Agricultural Engineering**, v. 39, p.25-33, 1997.

MCKENDRY, P. Energy production from biomass (part 2): conversion technologies. **Bioresource Technology**, v. 83. n. 1, p. 47-54, 2002.

MOOLER, H. B.; SOMMER, S. G.; AHRING, B. K. Methane productivity of manure, straw and solid fractions of manure. **Biomass Bioenergy**, v. 26, p. 485–495, 2004.

MOOLER, H. B.; MOSET, V.; BRASK, M.; WEISBJERG, R.; LUND, P. Feces composition and manure derived methane yield from dairy cows: influence of diet with focus on fat supplement and roughage type. **Atmospheric Environment,** v. 94, p. 36-43, 2014.

.

MOOLER, H. B.; SARKER, S.; HELLWIN, A. L. F.; WEISBJERG, M. R. Quantification of methane production and emission from anaerobic digestion of cattle manure derived from different feeding, 2012. Available from: <http://cigr.ageng2012.org/images/fotosg/tabla_137_C0327.pdf>. Accessed: 28 July 2016.

MONTGOMERY, S. P.; DROUILLARD, J. S.; SINDT, J. J.; GREENQUIST, M. A.; DEPENBUSCH, B. E.; GOOD, E. J.; LOE, E. R.; SSILPIZIO, M. J.; KESSEN, T. J.; ETHINGTON, R. T. Effects of dried full-fat corn germ and vitamin E on growth performance and carcass characteristics of finishing cattle. **Journal Animal Science**, v. 83, p. 2440-2447, 2005.

PRATT, C.; REDDING, M. R.; HILL, J. A promising and simple method to quantify soil/manure mixing on beef feedlot pens. **Animal Production Science**, v. 56, p. 1361-1366, 2016.

RIMKUS, R. R.; RYAN, J. M.; COOK, E. J. Full-scale thermophilic digestion at the West-Southwest Sewage Treatment Works, C. **Journal of the Water Pollution Control Federation,** v. 54, n. 11, p. 1447-14457, 1982.

SMITH, K.; GRYLLS, J.; METCALFE P.; JEFFREY, W.; SINCLAIR, A**. Nutrient value of digestate from farm-based biogas plants in Scotland**. Wolverhampton: ADAS UK, 2007. Report for Scottish Executive Environment and Rural Affairs Department. ADA/009/06.

SOOMER, S. G.; PETERSEN, S. O.; MOOLER, H. B .Algorithms for calculating methaneand nitrous oxide emission from manure management. **Nutrient Cycling in Agroecosystems**, v. 69, n. 2, p. 143-154, 2004.

TAMBONE, F.; SCAGLIA, B.; D'IMPORZANO, G.; SCHIEVANO, A.; ORZI, V.; SALATI, S.; ADANI, F. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. **Chemosphere**, v. 81, p. 577-583, 2010.

EPA (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY). **Method 1684**: total, fixed, and volatile solids in water, solids, and biosolids. Washington, DC: EPA, 2001. p. 16.

VAN SOEST, P. J.; ROBERTSON, J. B.; LEWIS, B. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. **Journal Dairy Science**. v. 74, p. 3583-3597, 1991.

ZAKTYS, P. I.; HOGAN, S. A.; O'SULLIVAN, M. G.; ALLEN, P.; KERRY, J. P. Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. **Meat Science**, v. 79, p. 648-655, 2008.

WILKIE, A.C. Anaerobic digestion of dairy manure: design and process considerations. In: **DAIRY MANURE MANAGEMENT:** TREATMENT, HANDLING AND COMMUNITY RELATIONS. Ithaca: Natural Resource, Agriculture, and Engineering Service; Cornell University, 2005. p. 301-312. (NRAES-176).