UNIVERSIDADE DE SÃO PAULO
Faculdade de Medicina Veterinária e Zootecnia
Departamento de Nutrição e Produção Animal

LAURA ALEXANDRA ROMERO SOLÓRZANO

**Interaction between monensin and lipids addition in diets as manipulators
of ruminal kinetics and fermentation for methane mitigation in ruminants**

Pirassununga

2019

LAURA ALEXANDRA ROMERO SOLÓRZANO

Interaction between monensin and lipids addition in diets as manipulators of ruminal kinetics and fermentation for methane mitigation in ruminants

Thesis submitted to the Postgraduate Program in Animal Nutrition and Production of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

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Prof. Paulo Henrique Mazza Rodrigues, Ph.D.

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CERTIFICADO

Certificamos que a proposta intitulada "Interação entre a adição de monensina e lipídeos como manipuladores da cinética e a fermentação ruminal para mitigação da produção de metano em ruminantes", protocolada sob o CEUA nº 9236160216, sob a responsabilidade de **Paulo Henrique Mazza Rodrigues e equipe; Laura Alexandra Romero Solorzano; Eduardo C. O. Cassiano; Flavio Perna Junior; Roberta Ferreira Carvalho** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilos Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 27/10/2016.

We certify that the proposal "Interaction between monensin and lipids as the kinetic handlers and ruminal fermentation to mitigate methane production in ruminants", utilizing 8 Bovines (8 females), protocol number CEUA 9236160216, under the responsibility of **Paulo Henrique Mazza Rodrigues and team; Laura Alexandra Romero Solorzano; Eduardo C. O. Cassiano; Flavio Perna Junior; Roberta Ferreira Carvalho** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 10/27/2016.

Finalidade da Proposta: **Pesquisa**

Vigência da Proposta: de **04/2016** a **08/2016**

Área: **Nutrição E Produção Animal**

Origem: **Prefeitura do Campus da USP de Pirassununga**

Espécie: **Bovinos**

sexo: **Fêmeas**

idade: **1 a 4 anos**

N: **8**

Linhagem: **Nelore**

Peso: **500 a 650 kg**

Resumo: O objetivo deste trabalho é avaliar a interação entre a adição de monensina e lipídeos como manipuladores da cinética e a fermentação ruminal, assim como da microbiota ruminal, para mitigação da produção de metano em ruminantes. Oito vacas canuladas no rúmen serão utilizadas e distribuídas a 8 dietas experimentais que diferirão quanto à fonte lipídica utilizada e à adição ou não de monensina sódica (30 ppm), sendo os tratamentos: 1) Controle (CON): dieta sem inclusão de lipídeo e sem monensina; 2) OC - SM: dieta com inclusão de 3,5% da MS de óleo de canola como fonte de ácido graxo saturado e sem adição de monensina; 3) OS - SM: dieta com inclusão de 3,5% da MS de óleo de soja como fonte de ácido graxo insaturado e sem adição de monensina; 4) OG - SM: dieta com inclusão de 3,5% da MS de óleo de girassol como fonte de ácido graxo insaturado sem fornecimento de monensina; 5) Controle com Monensina (CON - CM): dieta sem inclusão de lipídeo e com adição de monensina; 6) OC - CM: dieta com inclusão de 3,5% da MS de óleo de canola e com adição de monensina; 7) OS - CM: dieta com inclusão de 3,5% da MS de óleo de soja com adição de monensina e; 8) OG - CM: dieta com inclusão de 3,5% da MS de óleo de girassol e fornecimento de monensina. O delineamento experimental utilizado será um quadrado latino 4x4 replicado, em um arranjo fatorial 2 x 4 (n = 32 unidades experimentais). Cada período experimental será constituído de 29 dias, onde 20 dias serão destinados para adaptação às dietas experimentais. A partir do dia 21 até o dia 26 será avaliado o consumo de MS, a digestibilidade aparente total dos nutrientes, a taxa de passagem e a degradabilidade ruminal. No dia 26 ocorrerá a mensuração contínua do pH ruminal e será coletado conteúdo ruminal para quantificação dos produtos da fermentação (CH₄ e AGCC), bem como da concentração de N-NH₃ e, da contagem total e diferencial de protozoários. Para avaliar a dinâmica ruminal, o rúmen de cada animal será esvaziado em dias alternados (28º e 29º dias) de cada período experimental, sendo antes (0 horas) e 3 horas após a alimentação matinal. Espera-se que a interação entre fontes lipídicas e monensina apresentem efeitos sinérgicos ou aditivos, na diminuição da emissão de gases de efeito estufa por ruminantes.

Local do experimento: Setor de animais fistulados, laboratório de cromatografia e laboratório de bromatologia e nutrição animal

São Paulo, 29 de novembro de 2016

UNIVERSIDADE DE SÃO PAULO

FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

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DEDICATION

A Deus por todas as maravilhas que me dá no meu dia a dia.

Aos meus pais, Aura Solórzano e Roberto Romero, pelo seu apoio incondicional, amor, força gestora deste triunfo, por lutarem sempre junto comigo pelos meus sonhos, por nunca me deixarem desistir e me dar aquela voz de força para não me deixar derrubar por barreira nenhuma e por ser a base de toda minha vida. Agradeço pelas suas orações e a fortaleza que são na minha vida. Graças a vocês sou a pessoa que sou hoje em dia. Obrigada por tudo meus anjos, amo muito vocês!!!

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ABSTRACT

SOLÓRZANO, L. A. R. **Interaction between monensin and lipids addition in diets as manipulators of ruminal kinetics and fermentation for methane mitigation in ruminants** [Interação entre a adição de monensina e lipídeos como manipuladores da cinética e a fermentação ruminal para mitigação da produção de metano em ruminantes]. 2019. 201 f. Thesis (PhD in Animal Science) – Faculty of Veterinary Medicine and Animal Science, University of Sao Paulo, Pirassununga, 2019.

The present study aimed to evaluate the interaction between the addition of monensin and lipids in diets as manipulators of rumen kinetics and fermentation for methane production mitigation in ruminants. The manuscript describes the results of three experiments that evaluated the effect of eight treatments which differed on the lipid source used and the addition or not of monensin sodium. The treatments were: Control (CONT), diet without lipid and monensin inclusion, canola (CAN), soybean (SOY) and sunflower (SUN). The inclusion of lipid sources in the diet was 3.5% of DM as oil. Besides vegetable oils inclusion, each cow in a square daily received 300 mg (approximately 32 mg / kg DM) of sodium monensin (Rumensin® 200, Elanco Saúde Animal, Brazil) during the whole experimental period, delivered twice a day (150 mg at 08h00 and 150 mg at 16h00), mixed with the diet. The objective of the experiment I was to evaluate the effect of diet supplementation with vegetable oils and monensin in cattle on ruminal fermentation, methane production, microbial protein synthesis and total nutrient digestibility in Nellore cows. The experiment II was carried out with the objective to evaluate the effect of diet supplementation with vegetable oils and monensin on ingestive behavior and ruminal kinetics in Nellore cows. In experiment III, the objective was to evaluate the effect of the combination of monensin and vegetable oils on CO₂, CH₄ and N₂O production during the process of anaerobic biodigestion and the potential of biogas production. In experiment I, Methane emission was reduced by SOY treatment in 22.2%. Effect of interaction was observed for total SCFA, where with no monensin inclusion in diets, CAN treatment reduced this variable in 31.52%, when compared with control treatment. Whereas in the presence of monensin, soybean oil potentialized the response, leading to a reduction of 36.97%, of total SCFA. In Experiment II, potential degradability (PD, %) of DM and NDF were reduced, whereas non degradable fraction (Ind, %) of DM and NDF were increased by the addition of lipids in the diet. This was related to kt (%/h) of DM and NDF which decreased with the addition of lipid sources. In experiment III, biogas, methane (CH₄) and carbon dioxide (CO₂) production increased in CAN (21.8, 19.6 and

29.5%, respectively) compared to CONT treatment. Lipid sources in the form of oil, as well as the antagonistic or synergistic effect of the association between monensin and vegetable oils result in changes in ruminal fermentation variables. The present experiment suggests that lipid sources that have been described to decrease enteric CH₄ emissions result in increased biogas production and CH₄ production in biodigestion process.

Key words: Biodigestion. Methane. Microbial protein. Protozoa. Ruminal kinetics.

RESUMO

SOLÓRZANO, L. A. R. **Interação entre a adição de monensina e lipídeos como manipuladores da cinética e a fermentação ruminal para mitigação da produção de metano em ruminantes.** [Interaction between monensin and lipids addition in diets as manipulators of ruminal kinetics and fermentation for methane mitigation in ruminants]. 2019. 201 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2019.

O presente estudo teve como objetivo avaliar a interação entre a adição de monensina e lipídeos em dietas como manipuladores da cinética ruminal e fermentação para mitigação da produção de metano em ruminantes. O manuscrito descreve os resultados de três experimentos que avaliaram o efeito de oito tratamentos que diferiram sobre a fonte lipídica utilizada e a adição ou não de monensina sódica. Os tratamentos foram: controle (CONT), dieta sem inclusão de lipídios e monensina, canola (CAN), soja (SOJ) e girassol (GIR). A inclusão de fontes lipídicas na dieta foi de 3,5% da MS na forma de óleo. Além da inclusão de óleos vegetais, cada vaca em um quadrado, recebeu diariamente 300 mg (aproximadamente 32 mg / kg MS) de monensina sódica (Rumensin® 200, Elanco Saúde Animal, Brasil) durante todo o período experimental, administrada duas vezes ao dia (150 mg às 8:00h e 150 mg às 16:00), misturada com a dieta. O objetivo do experimento I foi avaliar o efeito da suplementação com óleos vegetais e monensina em bovinos sobre a fermentação ruminal, produção de metano, síntese de proteína microbiana e digestibilidade total de nutrientes em vacas Nelore. O experimento II foi realizado com o objetivo de avaliar o efeito da suplementação com óleos vegetais e monensina sobre o comportamento ingestivo e a cinética ruminal em vacas Nelore. No experimento III, objetivou-se avaliar o efeito da combinação de monensina e óleos vegetais na produção de CO₂, CH₄ e N₂O durante o processo de biodigestão anaeróbia e o potencial de produção de biogás. No experimento I, a emissão de metano foi reduzida pelo tratamento com SOJ em 22,2%. O efeito da interação observado para o total de AGCC, onde sem inclusão de monensina nas dietas, o tratamento com CAN reduziu essa variável em 31,52%, quando comparado com o tratamento controle. Enquanto na presença de monensina, o óleo de soja potencializou a resposta, levando a uma redução de 36,97% do total de AGCC. No Experimento II, a degradabilidade potencial (DP,%) da MS e FDN foi reduzida, enquanto a fração não degradável (Ind,%) da MS e FDN foi aumentada pela adição de lipídios na dieta. Isso foi relacionado à kt (% / h) da MS e FDN que diminuiu com a adição de fontes lipídicas. No experimento III, a produção de biogás, metano (CH₄) e

dióxido de carbono (CO_2) aumentou com o tratamento CAN (21,8, 19,6 e 29,5%, respectivamente) em comparação ao controle. Fontes lipídicas na forma de óleo, bem como o efeito antagônico ou sinérgico da associação entre monensina e óleos vegetais, resultam em mudanças nas variáveis de fermentação ruminal. O presente experimento sugere que fontes lipídicas que foram descritas para diminuir as emissões de CH_4 entéricas resultam em aumento da produção de biogás e produção de CH_4 no processo de biodigestão.

Palavras chave: Biodigestão. Cinética ruminal. Metano. Proteína microbiana. Protozoários.

SUMARY

1. INTRODUCTION	16
2. LITERATURE REVIEW	22
2.1 DRY MATTER INTAKE	23
2.1.1 Food consumption regulating factors	23
2.1.2 Chemical supplementation effect on the consumption of dry matter	25
2.1.3 Monensin supplementation effect on the consumption of dry matter	27
2.2 PARAMETERS OF RUMINAL KINETICS	28
2.2.1 Rate of passage	28
2.2.2 Ruminal degradability	29
2.2.3 Lipidic supplementation effect on the digestibility of dry matter and its fractions and on ruminal kinetics parameters	30
2.2.4 Monensin supplementation effect on the digestibility of dry matter and its fractions and on rumen kinetics parameters	31
2.3 RUMINAL FERMENTATION	33
2.3.1 Production and metabolic destiny of H ₂ in the rumen	33
2.3.2 Ruminal methanogenesis	34
2.3.3 Ruminal microbiota	35
2.3.4 Food strategies that alter ruminal fermentation and reduce methane emission	40
2.4 ANAEROBIC BIODIGESTION OF BOVINE RESIDUES	53
2.4.1 Parameters that interfere with the biogas production	54
2.4.2 Substrate importance in the biodigestion process	57
2.4.3 Stages and microorganisms involved in the biogas production process	59
3. DIET SUPPLEMENTATION WITH VEGETABLE OILS AND MONENSIN: EFFECT ON RUMINAL FERMENTATION, METHANE PRODUCTION, MICROBIAL PROTEIN SYNTHESIS AND NUTRIENT DIGESTIBILITY	83
3.1 INTRODUCTION	85
3.2 MATERIALS AND METHODS	87
3.2.1 Animals, Treatments and Experimental design	87
3.2.2 Dry matter intake, total tract apparent digestibility of dry matter and its fractions and nutrients excretion	91
3.2.3 Variables and products of ruminal fermentation	92
3.2.4 Rumen protozoa counts	97
3.2.5 Energy partition – Energy balance	97
3.2.6 Nitrogen compounds, microbial protein synthesis and nitrogen balance	99
3.2.7 Statistical analysis	102
3.3 RESULTS	103
3.3.1 Dry matter intake, total tract digestibility and nutrients excretion	103
3.3.2 Ruminal pH	106
3.3.3 Rumen fermentation products	108
3.3.4 Rumen protozoa	111
3.3.5 Energy partition	112
3.3.6 Nitrogen compounds, microbial protein synthesis and nitrogen balance	115

3.4 DISCUSSION.....	117
3.5 CONCLUSION	124
4. KINETICS OF RUMEN DEGRADABILITY AND PASSAGE RATE OF DRY MATTER AND NEUTRAL DETERGENT FIBER IN NELLORE COWS FED DIETS SUPPLEMENTED WITH VEGETABLE OILS AND MONENSIN.....	136
4.1 INTRODUCTION	138
4.2 MATERIALS AND METHODS	140
4.2.1 Animals, Treatments and Experimental design.....	140
4.2.2 Dry matter and water intake	144
4.2.3 Ingestive behavior.....	144
4.2.4 Percentage of particle size distribution and diet selection index.....	145
4.2.5 Ruminal kinetics variables.....	147
4.2.6 Statistical analysis	152
4.3 RESULTS	153
4.3.1 Dry matter and water intake	153
4.3.2 Ingestive behavior.....	153
4.3.3 Percentage of particle size distribution and diet selection index.....	155
4.3.4 Rumen kinetics variables.....	156
4.4 DISCUSSION.....	160
4.5 CONCLUSION	166
5. POTENTIAL OF BIOGAS PRODUCTION FROM WASTE FROM NELLORE CATTLE FED DIETS SUPPLEMENTED WITH VEGETABLE OILS AND MONENSIN	173
5.1 INTRODUCTION	175
5.2 MATERIALS AND METHODS	177
5.2.1 Animal feeding and feces and urine sampling.....	178
5.2.2 Substrate, treatments and experimental design	180
5.2.3 Measurement of production and sampling of biogas.....	182
5.2.4 Biogas composition	184
5.2.5 Measurement of methane, carbon dioxide and nitrous oxide gases production.....	184
5.2.6 Kinetics of biogas production.....	185
5.2.7 Laboratory analysis.....	186
5.2.8 Nutrients removal	186
5.2.9 Statistical analyses.....	187
5.3 RESULTS.....	188
5.3.1 Biogas production.....	188
5.3.2 Biodigestion and nutrients removal.....	188
5.4 DISCUSSION.....	191
5.5 CONCLUSION	196
REFERENCES	197

CHAPTER I.

INTRODUCTION

1. INTRODUCTION

Feed intake, passage rate and nutrient digestibility in gastrointestinal tract are variables that allow the determination of the true nutritional value of feed. In the same way, the estimation of the effective degradability of the dry matter becomes one of the most important tools in rumen kinetics study, since it allows to establish the quantity of each fraction degraded in the rumen (NRC, 2001). The knowledge of feed characteristics becomes an essential practice in order to meet the nutritional requirements of animals, exploring their maximum digestive capacity, in order to reach their genetic potential for the diet use (ROMERO, 2012). Although studies of ruminal kinetics and digestibility generate a great estimate of feed quality, it does not reflect feed characteristics in terms of energy efficiency and conversion into final product. Therefore, rumen fermentation study is important, as in the digestive process of microbial anaerobic fermentation, ruminants may or may not be efficient depending on the type of product generated: short chain fatty acids (SCFA) and methane (CH₄). Thus, ruminants are recognized as an important source of CH₄ emission into the atmosphere and the production of this gas, which may vary according to the feeding system, and is considered an energy loss, reflecting inefficiency in animal production (PEDREIRA et al., 2005). The knowledge of the factors that determine the efficiency of ruminal fermentation process and the emission of polluting products is essential for reduction of environmental impact caused by animal production systems (NASCIMENTO, 2007).

Overall, ruminant animals produce approximately 80 million tonnes of CH₄, which accounts for 28% of anthropogenic emissions annually (BEAUCHEMIN et al., 2008). Methane is a normal product of ruminal fermentation, which represents a pathway for elimination of metabolic hydrogen produced during microbial metabolism (McALLISTER; NEWBOLD, 2008). However, this greenhouse gas is considered a significant energy loss to the animal, estimated at 2 to 12% of the raw energy of feed (JOHNSON; JOHNSON, 1995). In this way, these energy losses can be reduced by improving productive efficiency through nutritional mitigation strategies (JOHNSON; JOHNSON, 1995; BOADI et al., 2004; MONTENY et al., 2006). Thus, when considering strategies to decrease rumen methanogenesis, we can mention the metabolic pathways involved in the formation and use of hydrogen (H⁺), as well as, the methanogenic population. Any strategy adopted should focus on one or more objectives, and it may be possible to predict several scenarios such as: the direct inhibition of methanogenesis with the necessity to divert H⁺ ions to

alternative products; the reduction of H^+ production in the rumen without damaging feed digestion; the stimulation of H^+ use of by alternative routes of beneficial products to the ruminant; the inhibition of methanogenic *Archaeas* (number and/or activity), with concomitant stimulation of pathways that consume H^+ to avoid the negative effects of partial pressure increase of H^+ in the rumen (McALLISTER; NEWBOLD, 2008; MARTIN et al., 2009).

From the what was exposed above, lipids become an excellent alternative to mitigate methane emissions, and different mechanisms of lipids mode of action are known, which contribute to reduction of the methanogenesis process. Among them, is the reduction of methanogenic activity by the presence of medium chain fatty acids (MACHMULLER et al., 2003; MACHADO et al., 2011); toxic effect on cellulolytic bacteria (NAGARAJA et al., 1997) and protozoa (DOREAU; FERLAY, 1995), exerted by unsaturated fatty acids and the biohydrogenation of polyunsaturated fatty acids (JOHNSON; JOHNSON, 1995), which contributes to increased propionic acid production in the rumen. For this latter mechanism, it has been attributed that fatty acids, mainly unsaturated, can fit as electron acceptors during the biohydrogenation process in the rumen (HEGARTY, 1999). In addition, long chain fatty acids generate a toxic effect on cell membrane, particularly on Gram-positive bacteria. Such changes in the ruminal microbial population favor propionic acid production, increasing H^+ uptake. Anti-methanogenic effect of ionophores, such as monensin, is more related to the inhibition of methane precursor formation (formate and H^+) than a direct effect on methanogenic population, since they are more resistant to ionophores than the bacteria that produce and supply H^+ (NAGARAJA et al., 1997).

There are many techniques for quantifying the individual or group emission of ruminal methane. However, some of these techniques require trained animals, a lot of work and have a high cost. Likewise, none of these methodologies can connect the products of the fermentation in the same material and at the same time. Thus, the ex-situ (micro-rumen) methane measurement methodology emerges as a viable technique that presents methane production in proportion to the other ruminal fermentation products. In the present project, the use of a viable and feasible technique to obtain new variables (relation between the production of SCFA and methane and the energy loss by methane emission relative to energy production via ruminal fermentation) in the same material and at the same time.

The objective with this experiment was to evaluate the association between the addition of monensin and lipids as manipulators of ruminal metabolism in cattle diet on ingestion (dry matter intake and ingestive behavior), digestion (*in vivo* total tract apparent digestibility and nutrient excretion), ruminal kinetics (ruminal passage rate, disappearance and degradability), ruminal fermentation (short chain fatty acids, methane, ruminal pH and ammoniacal nitrogen concentration), as well as, the characterization of rumen protozoa population and nitrogen and energy balance. Additionally, the objective with this study was to evaluate the effect of the combination of monensin and vegetable oils on CO₂, CH₄ and N₂O production during the process of anaerobic biodigestion as an alternative for bovine manure treatment (biofertilizer) and the potential biogas production.

It is expected that lipid sources, which can direct the energy from the rumen to the posterior digestive tract and have a direct effect on methanogenic microbiota, as well as, a small effect as a H⁺ drain, associated to monensin, which can direct the H⁺ generated from ruminal metabolism, may modify ruminal metabolism and fermentation variables, which will result in increased microbial protein synthesis, contributing to a substantial decrease in methanogenesis with a consequent reduction in enteric CH₄ production. Regarding the evaluation of the anaerobic biodigestion process, it is expected that the addition of sources of vegetable oils and monensin in diets will increase the reduction of solids and fibrous constituents, as well as, CH₄ emissions from cattle manure, as a result of the potentialization of the anaerobic biodigestion process and the decrease of N₂O formation in the substrate. Further, sources of unsaturated fatty acids, when combined with monensin, are expected to exhibit synergic effects (potentialized), or at least additive effects, more substantial in reducing greenhouse gas emissions from ruminants.

REFERENCES

- BEAUCHEMIN, K.A.; KREUZER, M.; O'MARA, F.; MCALLISTER, A.T. Nutritional management for enteric methane abatement: A review. **Australian Journal of Experimental Agriculture**, v.48, p.21-27, 2008.
- 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.830, p.319-335, 2004.
- 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.
- HEGARTY, R.S. Reducing rumen methane emissions through elimination of rumen protozoa. **Australian Journal of Agricultural Research**, v.50, p.1321-1327, 1999.
- JOHNSON, K.A.; JOHNSON, D.E. Methane Emissions from Cattle, **Journal Animal Science**, v.73, p.2483-2492, 1995.
- MACHADO, F.S.; PEREIRA, L.G.R.; GUIMARÃES, R.JR.; LOPES, F.C.F.; CHAVES, A.V.; CAMPOS, M.M.; MORENZ, M.J.F. Emissões de metano na pecuária: conceitos, métodos de avaliação e estratégias de mitigação / Fernanda Samarini Machado. [et al.], – Juiz de Fora: **Embrapa Gado de Leite**. 2011, 92p. (Embrapa Gado de Leite, Documentos, 147).
- MACHMULLER, A.; SOLIVA, C.R.; KREUZER, M. Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion, **British Journal of Nutrition**, v.90, p.529-540, 2003.
- MARTIN, C.; MORGAVI, D.P.; DOREAU, M. Methane mitigation in ruminants: from microbes to the farm scale. **Animal**, v.4, p.351-365, 2009.
- McALLISTER, T.A., NEWBOLD, C.J. Redirecting rumen fermentation to reduce methanogenesis. **Australian Journal of Experimental Agriculture**, v.48, p.7-13, 2008.
- MONTENY, G.J.; BANNINK, A.; CHADWICK, D. Greenhouse gas abatement strategies for animal husbandry. **Agriculture, Ecosystems and Environment**, v.112, p.163-170, 2006.
- NAGARAJA, 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, p.523-632, 1997.
- NASCIMENTO, C.F. **Emissão de metano por bovinos Nelore ingerindo Brachiaria brizantha em diferentes estádios de maturação**. Carolina Fernanda Moysés do Nascimento – Pirassununga: C.F.M. Nascimento. 2007, 65 p. Dissertação (mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2007.

NRC, NATIONAL RESEARCH COUNCIL. **Nutrient requirements of dairy cattle**. 7th ed, Washington: National Academies Press, 2001, 381p.

PEDREIRA, M.S.; OLIVEIRA, S.G.; BERCHIELLI, T.T.; PRIMAVESI, O. Aspectos relacionados com a emissão de metano de origem ruminal em sistemas de produção de bovinos. **Archives of Veterinary Science**, v.10, n.3, p. 24-32, 2005.

ROMERO, L.A. **Efeito de fontes energéticas sobre a fermentação ruminal, produção de metano determinada pela técnica do gás traçador SF₆, digestibilidade aparente total e excreção de nutrientes em bovinos**. 2012. 121 p. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2012.

CHAPTER II.

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 DRY MATTER INTAKE

The dry matter intake (DMI) is considered as one of the main factors correlated to the production of ruminants, because in the dry matter are located the nutrients necessary to meet the requirements of the animal organism (DIAS et al., 2008)..

2.1.1 Food consumption regulating factors

Food intake regulation is a complex mechanism. There isn't a single factor that could be considered as the sole regulator of intake. Instead, this process is controlled by a combination of different mechanisms that interact with each other (FISHER, 2002). One factor can stimulate several receptor types or a single receptor can be stimulated by a number of parameters (FORBES, 1995). In simple terms, food intake regulation can be given in short term or long term. One-day events that affect the frequency and size of meals are considered to represent the short-term regulation of food consumption, while long-term regulation describes average daily consumption over long periods of time during which maintenance needs will remain the same (MERTENS, 1987).

Diet consumption can be limited by the weight and volume of the food (filling effect) in relation to the volume of the reticulum-rumen (MERTENS, 1987; VAN SOEST, 1994; ALLEN, 2000; FISHER, 2002) being this, the characteristic of diet that describes the regulation of the intake by a physical effect. The rumen-reticulum volume of ruminants determines the physical potential of forage intake (FORBES, 1995). The energy content of the diet is the second most influential factor in the regulation of food intake (MERTENS, 1987; FORBES, 1995; ALLEN, 2000; FISHER, 2002). However, animal body weight (reticulum-ruminal capacity), its production potential (energy requirements for production) and health and physiological state (FORBES, 1995) are other associated characteristics that interfere with the animals' capacity to use the energy of a diet and that, consequently, affect the consumption of food (MERTENS, 1987).

The digestion of food to produce protein and energy to be used by the animal is a complex process. Many dietary factors are known to influence the availability of energy from consumed foods. Fisher (2002) affirms that a balance between different food

components is needed to ensure that the many nutritional factors involved in controlling voluntary consumption are appropriate.

A significant decrease in voluntary dry matter intake (DMI) was reported when protein was limited or when there was imbalance in dietary protein intake. This is probably explained by metabolic limitations for energy processing from carbohydrates in the rumen and post-rumen (FISHER, 2002). The palatability of the diet has the capacity to influence the voluntary consumption of food. In addition, the ambient temperature may also affect the voluntary consumption of dairy cows (FORBES, 1995). All these factors produce some kind of feedback signals through the central nervous system.

2.1.1.1 Regulation of consumption by physical effect

The reticulum-rumen represents the first chamber in the alimentary tract of ruminant animals and its capacity defines the limit for the amount that the animal can eat (FORBES, 1995). Anything that causes sufficient distention of the reticulum-rumen or any other compartment of the digestive tract stimulates the mechano-receptors to transmit a message to the central nervous system, resulting in cessation of increased intake (ALLEN, 2000). The regulation of food consumption by physical effect is performed based on the filling effect, which causes physical distention of the reticulum-rumen (MERTENS, 1987). According to Van Soest (1994), diet composition and physical form have a significant effect on filling and reticulum-rumen passage rate. Most intake regulation theories include the idea that food intake causes changes in the body, which are controlled by the central nervous system and used to determine when feeding should stop (FORBES, 1995).

Low quality or nutrient poor forages are usually characterized by slower ruminal digestion, longer retention times and a low passage rate, thus generating rumen-ruminal filling. Accordingly, forage grasses with a high rumen digestion rate have been associated with increased voluntary feed intake.

2.1.1.2 Regulation of consumption by metabolic effect

Short chain fatty acids (SCFAs) that are produced by the rumen microbiota account for up to 70% of the energy supply to the animal. The SCFAs are produced as a result of microbial degradation of foods in the rumen and are transported into the bloodstream through the portal vein hepatic. When the energy or protein needs of dairy cows are fully

satisfied by the diet, then the consumption of food is reduced, resulting in a "feedback" through the satiety center of the central nervous system. Boudon et al. (2007) suggested that the amount of energy absorbed in the form of SCFAs could be a strong limitation to voluntary food consumption. Apparently, diets containing high levels of concentrate or high quality silage tend to decrease food intake (FORBES, 1995; ALLEN, 2000). The mechanism explaining this is the high production of SCFAs in a very short period of time, due to the high rate of digestion in the rumen; where they are absorbed quickly into the bloodstream. Therefore, this increased level of SCFAs or energy entering the bloodstream depresses the consumption of food by a satiety effect produced in the ventro-medial hypothalamus (FORBES, 1995; ALLEN, 2000).

The main sensitive area to metabolic regulation of consumption in the digestive tract of ruminants is the reticulum-rumen, probably because it has epithelial receptors to detect products of ruminal metabolism, such as SCFAs. As SCFAs production increases, ruminal pH decreases (FORBES, 1995). The decrease in ruminal pH can affect the activity of the reticulum-rumen muscles, thus reducing the rumen motility, which in turn reduces the rumination process and, consequently, the rate of passage of food. When ruminal pH falls below 5,0, there is even more depression of food consumption, due to a ruminal atony generated (FORBES, 1995).

2.1.2 Chemical supplementation effect on the consumption of dry matter

Lipids incorporation into ruminant feed is commonly practiced in substitution of high proportions of grains, in order to increase the diet's energy density, also increasing the feed efficiency by the animal, because of its high energy value (CHILLIARD, 1993), which is readily available because lipids are composed of a large proportion of fatty acids, which are 2,25 times more energy than carbohydrates (SILVA et al., 2007). Thus, replacing high levels of grain, lipids avoid disorders caused by high starch intake (CHALUPA et al., 1984; PALMQUIST et al., 1993).

However, its use limitation and especially when supplied at levels higher than 7% of dry matter comes from: (1) a tendency to decrease milk protein content (WU; HUBER, 1994); (2) frequent changes in the ruminal fermentation process and (3) a decrease or negative effect on fiber digestion and reduction of ruminal degradability by rumen bacteria, which may require lipid protection against ruminal biohydrogenation (PALMQUIST; JENKINS, 1980; CUPPOCK; WILKS, 1991; JENKINS, 1993). In

addition, lipids contribute to the release of cholecystokinin (CCK), which inhibits dry matter consumption, since it affects gastric emptying (ALLEN, 2000). This decrease in feed intake when given lipids is given by a change in ruminal fermentation due to a reduction in digestibility of the fiber in the rumen which results in an increase in NDF retention time which results in increased ruminal filling (JENKINS; McGUIRE, 2006). Thus, when it is desired to provide lipids in the ruminant diet, it is important to evaluate its effects on the intake and digestion of nutrients, so as not to undermine the input needed for the desired production (PALMQUIST, 1994; JENKINS; McGUIRE, 2006).

Most foods present lipids used in animal feed, have in their constitution higher proportions of unsaturated fatty acids (VAN SOEST, 1994), which are toxic to ruminal microorganisms, mainly Gram-positive bacteria and protozoa, and adhere to the (JENKINS, 1993), which causes a decrease in the speed of digestion (ALLEN, 2000). The unsaturated fatty acids that reach the rumen undergo the process of hydrolysis of the triglycerides and, later, the biohydrogenation by the microorganisms of the rumen. However, lipids, when excess in the diet, can affect ruminal digestion and the greatest effect is the reduction of the digestibility of the fiber, as indicated previously, which results in a reduction of the methane production and the acetate: propionate ratio, if the levels of unsaturated fatty acids are high (HOBSON; STEWART, 1997; DOREAU; FERLAY, 1995). These effects may be due to reductions in the growth of bacteria (especially cellulolytic/Gram-positive) and protozoa (TAMMINGA; DOREAU, 1991).

Studies indicate that the inclusion of lipids in the diet may adversely affect the dry matter intake of cattle. However, the magnitude of this effect depends on the lipid source and degree of saturation. It should be noted that this effect tends to be greater when the bulk is supplied in the form of silage, mainly corn. Similarly, the reduction of dry matter intake after inclusion of lipids in the diet may be a result of: 1) an increase in rumination time due to the negative effects on digestion in the rumen, mainly of the fiber and; 2) a decrease in rumen emptying due to a metabolic effect generated by long chain fatty acids (LCFA) (CHILLIARD et al., 1993). In both situations, it is due to a satiety effect, either by physical effect or by metabolic effect.

Pantoja et al. (1994) observed a linear reduction of organic matter consumption (19,4 kg/d to 16,6 kg/d), combined with a linear reduction of neutral detergent fiber (NDF) digestibility (from 51,4% to 43,8%), presumably due to the degree of unsaturation of the added fat. In contrast, Beauchemin et al. (2009) observed a decrease in the digestibility of the organic matter in 18% without affecting the dry matter consumption.

The effect of the degree of lipid unsaturation was investigated by Bremmer et al. (1998), with an abomasum infusion of 450 g/d of a mixture of unsaturated lipid sources (18:1 + 18:2 + 18:3 = 70 g/d; 187 g/d; 200 g/d; 257 g/d, and 384 g/d) and observed that the dry matter consumption fell from 1,2 kg / d, with the most saturated fat mixture at 3,7 kg / d with the less saturated mixture, compared with the control treatment (22,8 kg/d). The authors reported that the negative effect observed may have been due to the secretion of chemical mediators in the duodenum. Litherland et al. (2005) observed no changes in plasma cholecystokinin concentration (CCK) as a result of fat abomasum infusion, but the reduction in dry matter intake was significantly related to the increase in plasma concentration of glucagon-like peptide-1 (GLP- 1). Nicholson & Omer (1983) suggest that unsaturated LCFAs may increase CCK secretion and thus inhibit reticulum-ruminal motility, which in turn may decrease DMC. According to Harvatine & Allen (2005), the reduction of dry matter intake when added unsaturated fatty acids in the bovine diet may be related to a satiety effect and a decrease in digestive motility as a result of the increase of peptide secretion intestinal.

2.1.3 Monensin supplementation effect on the consumption of dry matter

Monensin is an antimicrobial ionophore that selectively depresses or inhibits the growth of microorganisms in the rumen, which is why it is used as an important manipulator of the fermentation parameters of the rumen. The different modes of action probably result from the alteration of the flow of monovalent ions by the membrane of the Gram-positive bacteria causing their lysis, and consequently, providing alteration of the fermentation and the products of the microbial digestion (GONÇALVES et al., 2012). Although there are many biological responses described in the literature for monensin, its mechanism of action can be consolidated into seven effects: 1) increased production of short chain fatty acids; 2) the negative effect it can generate on food consumption; 3) reduction in the production of gas, methane; 4) handling of dry matter digestibility; 5) changes in protein utilization that appear to result from several factors occurring simultaneously; 6) influences the filling of the rumen and the rate of passage, both of which are determinants of some of the effects mentioned above and; 7) increased production and animal performance (SCHELLING, 1984).

Monensin's effect on dry matter intake in ruminants is inconsistent in the literature. The mechanisms by which ionophores promote decreased food intake are not yet fully

elucidated. However, the lower consumption is related to the greater use of dietary energy. This fact is linked to the changes in the concentration of short chain fatty acids (acetic, propionic and butyric) produced in the reticulum-rumen and the greater intestinal availability of peptides of origin feeding (LEITE, 2007). In a study by Sauer et al. (1989) reported a decrease in dry matter intake in cows treated with 33 ppm monensin during early lactation. Wagner et al. (1999) also indicated decreased dry matter intake during the whole lactation period when monensin was added at 16 and 24 ppm levels. On the other hand, in studies with animals under confinement conditions, fed with grain-rich diets supplemented with monensin, a food intake depression of 10.7% was observed (SCHELLING, 1984). However, consumption depression may be even greater when animals are abruptly supplemented without adaptation to monensin, which leads to a reduction of food consumption by up to 16% (SCHELLING, 1984). Owens, (1980) indicates for cattle fed with high-grain diets a food consumption depression of 5%. On the other hand, Schelling (1984) indicates for animals under grazing conditions a lower intake of food (3%). Opposite to the previous one, in a review of 12 studies, carried out by Ipharraguerre & Clark, (2003), did not observe any significant ionophore effect on dry matter intake.

2.2 PARAMETERS OF RUMINAL KINETICS

2.2.1 Rate of passage

The rate of passage or transit refers to the flow of undigested residues through the digestive tract. The ruminal flow includes, in addition to the indigestible fiber, bacteria and other undegraded fractions of the food, the composition and volume of the diet being external variables that influence the digestion rate and the recycling of ruminal content (VAN SOEST, 1994). The passage rate is an indication that fiber digestibility decreases as the rate of passage increases. This parameter is often expressed in retention time because it is intuitively easier to conceptualize digestibility in linear time function, rather than curvilinear flow rate function (ALLEN; MERTENS, 1988). Thus, it is important to understand that the retention time is exclusively a function of the passage rate only when the nutrient or label is indigestible. The retention time of the digestible nutrients in the rumen is a reciprocal function of their total disappearance rate and is not analogous to the retention time for passage.

The rate of passage may be affected by extrinsic or intrinsic factors. The extrinsic factors are related to the characteristics of animals and diet (HUHTANEN et al., 2006), while the intrinsic factors are related to the particle size of the food (RODRIGUE; ALLEN, 1960), particle size reduction rate (KUOPPALA et al., 2009), ruminal motility (SISSONS et al., 1984) and the specific gravity (EHLE, 1984), which are determined by the type of feed (forage vs. concentrate, type of forage, and species) and stage of maturity (LUND, 2002; KUOPPALA et al., 2009, 2010). Thus, any of these factors could alter the digestibility through its effect on the passage rate. On the other hand, the flow rate of rumen fluids may vary according to the level of intake, the proportion of concentrate, the indicator used, as well as the sampling sites in the rumen (OWENS; GOETSCH, 1988).

ELLIS et al. (1983) stated that an appropriate indicator used to determine the rate of passage should behave exactly like the food particle, not altering the normal process of mixing and flow. To estimate the flow of the fraction digested through the compartment, the indicator should adhere to the particle of the undigested residue and not influence the flow of the labeled particle, compared to the unlabeled particle.

2.2.2 Ruminal degradability

The in situ ruminal degradation technique has been widely adopted to evaluate the rate and extent of rumen food degradation (CORREA, 2008). The determination of the effective rumen degradability (ED) is perhaps the most important calculation in the ruminal kinetics study since it allows to establish the proportion of nutrient fractions that are degraded and those that can not be degraded in the rumen (NRC, 2001). In other words, the determination of nutrient degradability allows us to evaluate the factors that affect the process of ruminants' food utilization. Orskov & McDonald (1979) described an exponential model to estimate the rumen degradability of nutrients as a function of rumen residence time based on data obtained by the in situ degradation technique: $RDb_t = a + b(1 - \exp^{-k_d t})$, where RDb_t is the ruminal degradability of fraction "b" at time t; "a" is the soluble fraction which is completely degradable in the rumen; "b" is the potentially degradable fraction in the rumen; "kd" is a constant and "t" is the residence time in the rumen.

The process of degradation and the transit of particles through the gastrointestinal tract are affected by factors inherent to the animal and the intrinsic characteristics of the food. These factors should be considered in mathematical models to more accurately

estimate the kinetic parameters of food degradation in the digestive tract. In the same way, it is important to point out that the adjustment of the models varies according to the fermentation environment and the nature of the substrate under study (forages vs. concentrate). In general, in degradability and in situ digestibility tests, these parameters can be directly affected by the incubation times selected to construct the curve and the number of experimental units used for this type of studies (ROSETO; POSADA, 2007).

As the differences in the characteristics of food degradation affect the dynamics and nutrient balance available for rumen microorganisms (McCARTHY et al., 1989), the study of ruminal degradation kinetics of different foods has shown results that allow nutritionists the balance of rations, aiming to maximize energy efficiency and protein utilization of microorganisms and ruminant (ANDRADE, 1994).

2.2.3 Lipidic supplementation effect on the digestibility of dry matter and its fractions and on ruminal kinetics parameters

Lipids when added to feed ruminants can significantly affect ruminal fermentation, causing a reduction in degradability and digestibility, especially of fiber. The digestion of the structural carbohydrates in the rumen can be reduced by 50% or more (IKWUEGBU; SUTTON, 1982; JENKINS; PALMQUIST, 1984). This reduction in digestion is accompanied by a decrease in the production of methane, hydrogen ions, and short chain fatty acids, including the acetate: propionate ratio (BOGGS et al., 1987; CZERKAWSKI; CLAPPERTON, 1984; IKWUEGBU; SUTTON, 1982). When lipids decrease ruminal fermentation, fermentation in the large intestine is limited, which may decrease fiber digestibility throughout the digestive tract (BOGGS et al., 1987; JENKINS, 1988).

Studies indicate that lipid inclusion in the diet of ruminants influences the digestibility and degradability of dry matter. Palmquist & Jenkins (1980), suggest a tolerance of 4 to 5% of lipid supplementation in order not to affect the dry matter degradability. On the other hand, in a study carried out by Messana et al. (2013), when evaluating different levels of lipid inclusion (2, 4 and 6% of DM) indicated that for animals fed 4% of lipids, there was a reduction of the potentially degradable fraction (b) of the dry matter of the silage in comparison to other treatments. Regarding the actual degradability of corn silage, this was higher when the animals received a diet with 6% of lipids, however, this may have been due to the adjustment mode of the mathematical model used to evaluate the results. On the other hand, these authors indicate that, although no

significant difference in the degradability of NDF was observed, a significant decrease ($P = 0.02$) in the potentially degradable insoluble fraction (b) occurred, being this effect proportional to the increase of the level of inclusion of unsaturated fatty acids in the diet. This indicates that the mechanisms by which unsaturated fatty acids influence the degradability of DM occurs independently of the absorption of bacteria and food particles, since the degradation of NDF has not been altered (MESSANA et al., 2012). Balieiro Neto & Melotti (2007) mention that the dissociation of lipids in the rumen and their effects on ruminal degradability are closely related to the pH of the rumen, the diet components and their level of intake.

Messana et al. (2012) indicated that, for the DM degradability of soybean meal, this was higher when the animals received 6% of lipid inclusion. However, for the potential degradation evaluated, it was higher for the treatment with 2% of lipids in relation to the treatments with 4 or 6% of lipids in the diet. A similar effect was found by Jacob et al. (2012), when they added 6% of sunflower and soybean oil, reducing the degradability of DM, NDF, ADF and hemicellulose compared to the control treatment for *in-vitro* evaluation. Jalc & Ceresnakova (2001) also used a high level of oil inclusion in an *in-vitro* study, observed depression in the degradability of DM. On the other hand, Bateman & Jenkins (1998), using soybean oil at increasing levels of 0, 2, 4, 6 and 8% of total DM in non-lactating cows, did not observe any effect on total apparent digestibility of DM, OM or NDF in a diet high in fiber using hay as a source of bulking. The authors indicated that high fiber level in the diet promoted rapid lipolysis and complete biohydrogenation of soybean oil, which altered any deleterious effect of soybean oil on ruminal digestibility. It is important to note that ruminal fermentation and degradation of DM and nutrients is compromised by an inclusion level of 6% of lipid sources (JACOB et al., 2012; MESSANA et al., 2012), since this level of inclusion may modify the microbial population or inhibit its activity (DEVENDRA; LEWIS, 1974).

2.2.4 Monensin supplementation effect on the digestibility of dry matter and its fractions and on rumen kinetics parameters

As for studies related to the effect of monensin on parameters of rumen kinetics, data related to rumen filler and passage rate are found, knowing that these two parameters play an important role in ruminant nutrition, because they influence the extension and the

digestion site, the extent of microbial fermentation, the final fermentation products and the use of nitrogen (SCHELLING, 1984).

Lemenager et al (1978); Pond & Ellis (1981); Ellis & Delaney (1982) indicate that monensin decreases the turnover rate of dry matter in the rumen and increases ruminal filling, however, the implications of these effects have not yet been clarified. This demonstrates that there is still insufficient data to firmly establish this relationship and, on the other hand, the effect of monensin on ruminal kinetic parameters should be further investigated.

In a study by Osborne et al. (2004) found that monensin supplementation (22 mg/kg DM) on ruminal degradability and forage digestibility in lactating cows induced by subacute ruminal acidosis, found that monensin did not affect ($P > 0,05$) the ruminal degradability of DM and NDF at 24 and 48 hours of forage incubation. According to this, monensin has been indicated to attenuate subacute ruminal acidosis in beef cattle (COOPER; KLOPFENSTEIN, 1996) and dairy cattle (GREEN et al., 1999) favors the ruminal environment because it allows the proliferation of cellulolytic bacteria by increasing ruminal pH, thus avoiding a decrease in fiber digestion, when metabolic disorders such as acidosis are present (KRAJCARSKI-HUNT et al., 2002).

On the other hand, in relation to the digestibility of DM and nutrients, the literature indicates that the influence of monensin sodium on this parameter in the rumen or post-ruminal is not consistent. The use of monensin supplementation reduced the digestibility of nutrients in the rumen in some studies with cattle or sheep (OWENS et al., 1978; SIMPSON, 1980; MUNTIFERING et al., 1981), but not in others (MORRIS et al., 1990; ROGERS et al., 1991). Osborne et al. (2004), did not observe effect of monensin on ruminal digestibility of the fiber. Likewise, the authors indicate that total digestibility of DM, BP, EE, and BE were not affected by the dietary addition of monensin. In contrast, Haïmoud et al. (1995) observed that monensin supplementation altered the digestibility of nutrients in the diet, reducing the ruminal digestibility of the fiber. However, post ruminal digestibility (small intestine) of the fiber was higher with monensin supplementation. According to the above, the reasons for these discrepancies between the studies may be related to differences in the levels of inclusion of monensin in the diet and the interactions between the ingestion and the composition of the food and the monensin (MUTSVANGWA et al., 2002).

2.3 RUMINAL FERMENTATION

2.3.1 Production and metabolic destiny of H² in the rumen

In the rumen, methane formation is the main form of hydrogen removal from all the chemical reactions that occur in the rumen (MOSS et al., 2000), through the reaction: $\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$.

Moss et al. (2000) indicate that metabolic hydrogen in the form of reduced protons (H) can also be used during the synthesis of short chain fatty acids or incorporated into the microbial organic matter. The stoichiometry of the main anaerobic fermentation pathways can be summarized in H₂-producing reactions: Glucose $\rightarrow$ 2-pyruvate + 4H glucose (Embden-Meyerhof-Parnas pathway or Glycolysis); Pyruvate + H₂O $\rightarrow$ C₂ + CO₂ + 2H; or user reactions of H₂: Pyruvate 4H $\rightarrow$ C₃ + H₂O; 2C₂ + 4H $\rightarrow$ C₄ + 2H₂O and CO₂ + 8H $\rightarrow$ CH₄ + 2 H₂O.

In general, ruminal degradation of fiber and starch yields hexoses which during fermentation follow the Embden-Meyerhof route or glycolysis, as mentioned previously. Pyruvate obtained in glycolysis is converted into short chain fatty acids (SCFA), mainly acetic, propionic and butyric acids, through different metabolic pathways. In this case, acetyl-CoA produced from pyruvate by phosphoroclastic reactions is metabolized to acetate via acetyl-phosphate or to butyrate via acetoacetyl-CoA. The total production of NAD in its reduced form (NADH+H⁺), H₂ and CO₂ by the glucose molecule fermented to acetate or butyrate is: 2NADH + 2H⁺, during glycolysis, and 2H₂ + 2CO₂, during decarboxylation of pyruvate, catalyzed reaction by the enzyme pyruvate ferredoxin oxidoreductase adapted to a hydrogenase. In this balance, an NADH + H⁺ molecule is oxidized during the reduction of acetoacetyl-CoA to butyrate (KIM and GADD, 2008). Species of the genus *Propionibacterium*, *Clostridium propionicum* and *Megasphaera elsdenii* ferment glucose or lactate to propionate, acetate and CO₂ (3 glucose $\rightarrow$ 4 propionate + 2 acetate + 2 CO₂; 3 lactate $\rightarrow$ 2 propionate + acetate + CO₂), reactions that arise through acrylate or succinate-propionate, which do not produce H₂ and which, on the contrary, consume.

The H₂ produced during the glycolysis is detrimental to the fermentation process, since its accumulation inhibits the enzyme NADH + H⁺ ferredoxin oxidoreductase, preventing the regeneration of NAD⁺, which is possible only at low H₂ pressure

(HEGARTY; GERDES, 1998). Therefore, the continuous production of CH_4 represents, as mentioned above, one of the mechanisms by which the H_2 rumen pressure decreases.

It should be pointed out that the preferential metabolic pathway of cellulolytic bacteria contributes notoriously to ruminal methanogenesis through H_2 interstitial transfer. The relationship between H_2 utilization and cellulolytic activity is explained by the fact that most of $\text{NADH} + \text{H}^+$, which forms during glycolysis by glyceraldehyde-3-phosphate oxidation, easily transforms into H_2 and NAD^+ when the partial pressure is low ($P_{\text{H}_2} < 1 \times 10^{-3}$ atm). Thus, more pyruvate is available to be oxidized to acetate and CO_2 via the acetyl pathway coenzyme A, with the generation of one mole of ATP per mole of acetate formed (LAUBE; MARTIN, 1981).

The relationship between methanogens and protozoa is another example of H_2 transfer between ruminal species. It has been described the presence of methanogenic microorganisms on the surface of ciliated protozoa of the rumen (VOGELS et al., 1980) and as endosymbionts within them (FINLAY et al., 1994). Newbold et al. (1995) estimated that the relationship between methanogenic and ciliate protozoa is responsible for 9 to 25% of methanogenesis in ruminal fluid. Since in the rumen aerobic respiration is not possible, protozoa do not contain mitochondria, but rather, intracellular organelles called hydrogenosomes, where the H_2 produced during the oxidation of pyruvate or malate (MÜLLER, 1993) is released. There is a close relationship between the methanogenic and the hydrogenosomes. The advantage of this cooperation for the protozoan is evident: the elimination of H_2 allows the protozoan to ferment organic matter to acetate and CO_2 , avoiding the generation of reduced products such as ethanol and lactate, obtaining a maximum production of ATP (SCHINK; STAMS, 2006).

2.3.2 Ruminal methanogenesis

In the rumen there are mainly three substrates for methanogenesis: CO_2 , methyl and acetate compounds. The methanogenic microorganisms use mainly H_2 to reduce CO_2 to CH_4 in a series of reactions adapted to the synthesis of ATP (LEAHY et al., 2010), where CO_2 is used as carbon source and H_2 as the main electron donor. The formate is also an important electron donor and may contribute 18% of CH_4 produced in the rumen (HUNGATE, 1970). Similarly, CH_4 can be produced from the acetate through the acetic-lithic pathway, but this metabolic pathway is only used by microorganisms of the order Methanosarcinal (IWAMOTO et al., 1999), which use acetate as carbon source and energy

for the production of CH₄. In addition, acetate is rapidly absorbed and used by the ruminant, with minimal participation in total methane production.

It should be noted that some variables influence methane production in ruminants. Among them, nutritional factors, which are related to the amount and type of carbohydrates in the diet, level of food intake, presence of lipids; metabolic factors, such as the rate of passage of digesta; environmental factors; animal management; beyond the physiological state; body size and, mainly, the population ruminal microorganisms such as protozoa and bacteria (PRIMAVESI et al., 2004). Therefore, the use of larger quantities of bulky foods, taking into account their quality, may have an impact on the production of methane by cattle.

2.3.3 Ruminal microbiota

The microbial population that inhabits the rumen is extremely diverse. Accordingly, different types of anaerobic bacteria are found inside the rumen, in addition to protozoa, fungi, methanogenic *Archaeas* and bacteriophages which contribute to the functioning of the ruminal ecosystem. These microorganisms play essential metabolic functions for the development, health and nutrition of the ruminant animal (MORGAVI et al., 2010).

The rumen is an anaerobic and methanogenic medium in which the degradation of organic matter is accomplished by the symbiotic cooperation of different groups of anaerobic microorganisms. The structural carbohydrates, proteins and other organic polymers contained in foods ingested by the animal are degraded to their monomeric components by primary anaerobic fermenters. These monomers are then converted to SCFA, CO₂ and H₂, both by primary fermenters and by microorganisms that do not have the ability to hydrolyse complex polymers by themselves (secondary fermenters) (MORGAVI et al., 2010). The methanogenic microorganisms are at the end of this trophic chain and use the final fermentation products as substrates. The synthesis of methane contributes to the efficiency of the system in that it avoids increasing the partial pressure of H₂ at levels that may inhibit the normal functioning of the microbial enzymes involved in electron transfer reactions, particularly NADH dehydrogenase, resulting in accumulation of NADH and, consequently, in reducing the ruminal fermentation process. The uptake of H₂ produced by one microbial species to another is usually referred to as the transfer of H₂ interest species (WOLIN et al., 1997).

2.3.3.1 *Methanogenic Archaeas*

Methanogenic *Archaeas* represent a unique group of microorganisms. These microorganisms have three coenzymes that have not been found in other microorganisms, such as: coenzyme 420, involved in the transfer of electrons in place of ferredoxin; coenzyme M, involved in the methyl transfer, and factor B, low molecular weight component, thermally stable and sensitive to oxygen coenzyme and involved in the enzymatic formation of CH₄ from methyl M coenzyme (JONES et al., 1987; BAKER, 1999). Methanogenic microorganisms in all habitats differ from almost all bacteria by cell membrane composition: there is no murane acid in the cell wall and cell membrane lipids are composed of isopranyl ether bound to glycerol or other carbohydrates (BAKER, 1999). These microorganisms are nutritionally anaerobic and grow only in environments with a redox potential below -300 mV (STEWART; BRYANT 1988). Most methanogenic microorganisms grow at neutral pH, ranging from 6 to 8 (JONES et al., 1987). These represent a group of polyphyletic microorganisms, comprising three orders, with eight families and 21 genera. They present morphology common to prokaryotic cells, in the form of bacilli of different sizes, cocci and filaments. Some representatives have Gram-positive and other Gram-negative staining properties, the taxonomy being based essentially on molecular methods, by comparison of 16SrRNA sequences. Morphophysiological analyzes facilitate the primary classification related to gender (SOWERS, 1995).

According to Kamra (2005), seven different species represent the five genera of methanogens that have been found in the rumen: *Methanobrevibacter ruminantium*, *Methanobacterium bryanti*, *Methanobacterium vibacter smithii*, *Methanoculleus olentangyi*, *Methanobacterium formicicum*, *Methanosarcina barkeri* and *Methanomicrobium mobile*. The methanogenic species most commonly isolated from the rumen are strains of *Methanobrevibacter*, *Methanomicrobium*, *Methanobacterium* and *Methanosarcina* (JARVIS et al., 2000).

Methanogenic microorganisms use the CH₄ formation process to generate energy for their own growth. The substrates used in this process include H₂, CO₂, formate, acetate, methanol, methylamine, dimethylsulfide and some alcohols (JONES, 1991; McALLISTER et al., 1996; LIU; WHITMAN, 2008). In the rumen, methanogenes mainly use H₂, CO₂ and formate as substrates for the process of methanogenesis (JONES, 1991). However, the predominant route of these microorganisms is hydrogenotrophic in which they use CO₂ as carbon source and H₂ as the main electron donor (HUNGATE, 1967). The formate is also

an important electron-donor pathway used by many hydrogenotrophic methanogenic microorganisms in the rumen and may represent up to 18% of the ruminal methane produced (HUNGATE et al., 1970). On the other hand, the unique biochemical capacity of *Methanosarcina barkeri* is in using methanol, methylamine, as well as CO₂ and H₂ as substrates that allow slow growth of these microorganisms in ruminants fed rations containing ingredients such as molasses that break down into methylamines, methanol and acetate (VICINI et al., 1987). Finally and as indicated above, methane is also produced from the acetate through the acetoclastic route, this route being also limited for the *Methanosarcina barkeri* species (LIU; WHITMAN, 2008).

Although methanogens are only directly involved in the final stages of fermentation, they are very important because they are able to efficiently use electrons in the form of H₂ to reduce CO₂ in CH₄, thus maintaining low H₂ pressure in the rumen. However, since CH₄ has no nutritional value, its production represents a loss of energy in the diet for the animal (JOHNSON; JOHNSON, 1995). The methanogenic microorganisms are found associated to both the liquid phase and the solid phase of the rumen, as well as to the ruminal epithelium. Information on the latter group is still scarce (PEI et al., 2010), however, the rumen epithelium appears to have a high concentration of methanogenic microorganisms and some new species of these microorganisms (PEI et al., 2010).

2.3.3.2 Ruminal protozoa

Morphologically, more than 250 ciliate species have been described in the ruminal ecosystem (WILLIAMS; COLEMAN, 1992). The large biomass of protozoa that exists in the rumen (40 to 80% of total microbial mass) (HARRISON; McALLAN, 1980) and its ability to attack the main food components (COLEMAN, 1985) suggest that although they are not essential in the ruminal medium, they play an important role in ruminal fermentation. Accordingly, the ciliate protozoa of the rumen are metabolically active, capable of influencing the ruminal fermentation process, as well as other microbial populations of the rumen and, consequently, affect the amount and proportion of the final products of the fermentation process, including the methane (WILLIAMS; COLEMAN, 1992; EUGENE et al., 2004).

The presence or absence of ciliates has been observed as a factor affecting ruminal parameters such as: pH, ammonia concentration, volume and dilution rate, dry matter of ruminal content, as well as the number and type of bacteria, which consequently, may

affect the rate and extent of food digestion (VEIRA, 1986). When using diets comparing defatted or ciliates, it was observed that the pH of the rumen contents was lower in ciliates-free animals (WHITELAW et al., 1972; JOUANY; SENAUD, 1982; VEIRA et al., 1983). On the other hand, ruminal pH was moderated by ciliary microorganisms (VEIRA et al., 1983) in animals with protozoan presence and fed diets containing high starch. This is due to the absorption capacity of rapidly fermentable sugars and starch by protozoa (OXFORD, 1955).

The results of Kurihara et al. (1968) and Jouany et al. (1981) suggest that the increase in bacterial mass occurring in the defaunation may not be sufficient to compensate for the loss of protozoan biomass. The lowest ruminal pH associated with the absence of protozoa may be one of the main reasons for the decrease in ruminal digestibility. Accordingly, in relation to the lower ruminal digestibility in defatted animals, this is partially compensated by a higher post ruminal digestion, resulting in less pronounced differences in terms of total digestibility of the tract for the different nutrients. A compensatory factor of defaunation could be a change in the fermentation process that results in energetically more efficient reactions in the rumen (less methane emissions), and lower metabolic energy required by the defaunted animals to eliminate excess urea in the ruminal environment as a result of reduction of microbial protein synthesis and ammonia levels in the rumen. Despite this compensatory mechanism in defaunted animals, the reduction of ruminal digestibility of nutrients is probably a disadvantage, since this may limit food intake and feed efficiency (HRISTOVETAL, 2013).

Concerning the total concentration of products of the ruminal fermentation process, these also differ in animals with presence or absence of ciliates (defaunted). When the fermentation rate is reduced, the protozoa contribute to the maintenance of a stable ruminal fermentation, avoiding the accumulation of excessive levels of lactate (MACKIE et al., 1978). The stabilizing effect of protozoa is an important factor in the adaptability of the rumen ecosystem when grains are replaced by forage, as well as in the prevention of acidosis (MACKIE et al., 1978). Regarding the concentration of ammonia in the rumen, this is consistently higher in animals with ciliates than in defatted animals. This is probably due to increased recycling of microbial protein into the rumen, since few bacteria use ammonia, and increase dietary protein degradation (LENG; NOLAN, 1984). According to this, a decrease in rumen ammonia is probably one of the most consistent effects of the defaunation process, and appears to be due to the decrease in microbial protein synthesis and food protein degradation in absence of rumen protozoa (WILLIAMS; COLEMAN,

1992). In addition, it has been demonstrated that, although bacterial predation by protozoa of the rumen is dependent on the size of the protozoan, holotricos protozoa have a much lower predatory activity than the entodiniomorphs (BELANCHE et al., 2012) and, finally an impact lower than the concentration of ammonia in the rumen (BELANCHE et al., 2015) and duodenal microbial protein flux (IVAN et al, 2000; IVAN, 2009). On the other hand, studies indicate that for lactating animals lactate levels are higher than in animals with ciliates (WILLIAMS; COLEMAN, 1992), because protozoa consume lactate faster than bacteria (NEWBOLD et al., 1986).

Based on the stoichiometry of anaerobic fermentation, high levels of SCFA result in decreased CH_4 production, since less H_2 will be available as a substrate for methanogenesis (DEMEYER et al, 1996). However, the methanogenesis process has also been related to the presence of protozoa in the rumen, this can be explained, because the methanogenic *Archaeas* have been observed attached to ciliated protozoa (VOGELS et al., 1980). In some studies, it has been reported that the number of protozoa and methanogenic *Archaeas* are reduced at the same time as the ruminal pH decreases. These observations imply a possible indirect role of protozoa in the formation of CH_2 (VEIRA et al., 1983). *In vitro* studies (DEMEYER; VAN NEVEL, 1979) and *in vivo* (EADIE; GILL, 1971; WHITELAW et al., 1984) show a higher production of CH_4 when protozoa are present in the ruminal environment. However, in other studies, no differences in CH_4 production have been observed (KRUMHOLZ et al., 1983; ITABASHI et al., 1984). In general, HEGARTY (1999) indicates that methane-mediated *Archaeas* attached to ciliate protozoa in the rumen can account for up to 37% of ruminal methane emissions. However, Morgavi et al. (2010) mentions that the variability of methane emissions may be responsible for ciliate microorganisms in 47%, indicating that methanogenesis is a complex process in which several microorganisms are involved. A number of mechanisms by which protozoa may influence the process of methanogenesis is based on their ability to produce H_2 in their hydrogenosomes (an organelle similar to mitochondria), on the ability to host endosymbiotic methanogenic microorganisms and to protect them from oxygen toxicity (FENCHEL; FINLAY, 2006). This interaction is a typical example of the transfer of interspecies H_2 which favors methanogenic microorganisms, as well as protozoa (USHIDA et al., 1997). On the other hand, it should be noted that in the absence of protozoa, CH_4 emissions from the rumen are reduced by an average of 13%, but this varies with the type of diet. Thus, the reduction of CH_4 emissions by protozoan free animals may be a consequence of reduced ruminal digestion of dry matter; the decrease of the population of

methanogenic *Archaeas*; the alteration of the production pattern of short-chain fatty acids, as well as the availability of hydrogen and the increase of oxygen partial pressure in the rumen (HEGARTY, 1999).

A number of authors have studied the symbiotic relationship between protozoa and methanogenic *Archaeas* (SHARP et al., 1998; IRBIS; USHIDA, 2004; TYMENSEN et al., 2012) and most of them indicate that *Methanobrevibacter sp.* the predominant genus among endosymbiotic microorganisms, whereas the contribution of *Methanomicrobium sp.* and the *Methanomassiliicoccales* for the community of endosymbiotic methanogens is variable and may indicate differences between the protozoan groups present in the ruminal environment (NEWBOLD et al., 2015). Recently, it has also been demonstrated that holotric protozoa have different endosymbiotic methanogenic microorganisms than entomopyodoform protozoans (BELANCHE et al., 2014), possibly because holotricos protozoa have more active hydrogensomes than entodiniomorphs (PAUL et al., 1990) and a rapid synthesis of glycogen, since in the presence of excess carbohydrates, they generate more hydrogen (HALL, 2011). These differences may explain the greater impact of holotric protozoa in the process of ruminal methanogenesis in relation to entodiniomorphs (BELANCHE et al., 2015).

Recent studies using molecular techniques have demonstrated that the process of defaunation also modifies the structure of the bacterial community, leading to a reduction in population structure and lower bacterial diversity (BELANCHE et al., 2012b, 2015). Newbold et al. (2015) observed a decrease in the concentration of fibrolytic microorganisms, such as anaerobic fungi (-92%, $P < 0.001$), *Ruminococcus albus* (-34%, $P < 0.001$), and *Ruminococcus flavefaciens* (-22%, $P < 0.001$) were observed in the absence of anaerobic fungi of protozoa. This observation suggests that the digestion of the fiber in the rumen is a complex process that requires the symbiotic collaboration of several fibrolytic microorganisms, including rumen protozoa, to perform the initial stages of colonization and fiber digestion.

2.3.4 Food strategies that alter ruminal fermentation and reduce methane emissions

In terms of rumen manipulation, three strategies to mitigate methane emissions can be considered: 1) reduction of H_2 production; 2) search for alternative drains for the H_2 already produced in the enteric fermentation and 3) reduce the populations of methanogenic microorganisms (JOBLIN, 1999). For this, in nutritional terms three basic

approaches are based: 1) the selection of ingredients in a diet that changes the production pattern of SCFA; 2) increased passage rate, which can alter microbial populations, the production of SCFA, as well as change the digestion of food and 3) the use of better quality diets to increase milk production per cow, which will dilute the cost associated with CH₄ with the maintenance energy needs (KNAPP et al., 2014).

Grainger & Beauchemin (2011) elaborated a review on nutritional and management strategies to reduce enteric methane and evaluate the potential effects on animal production. These strategies include intensive pasture management, grain use and concentrated feed, forage processing and conservation to reduce particle size and increase digestibility, increase the use of legumes by exploring the presence of tannins and saponins, the use of essential oils, the addition of saturated and unsaturated lipids, as well as additives such as ionophores, nitrate, yeast, malate and fumarate. It should be noted that for a successful mitigation strategy, this should enable a profitable increase in milk and / or meat production, as well as promote a persistent reduction of enteric methane emission (GRAINGER et al., 2010).

2.3.4.1 Effect of lipids on ruminal fermentation parameters

In the rumen the lipids of the diet undergo the hydrolysis process by the action of the microbial lipases and then, the biohydrogenation of the unsaturated fatty acids occurs by the bacteria of the rumen. In the case of linoleic acid (C18:2 n-6), the final product of biohydrogenation is stearic acid (C18:0) (JENKINS, 1993). When biohydrogenation is incomplete, the intermediates (eg, trans-18: 1) of this process are available for deposition in microbial biomass as well as in body tissues (BAUMAN; GRINARI, 2003).

The different effects of lipids on the ruminal fermentation process are generally attributed to some basic differences in their lipid structure. A factor is the degree of unsaturation, because unsaturated fatty acids generate more changes in the fermentation process than saturated fatty acids (CHALUPA, et al., 1984; PALMQUIST; JENKINS, 1980). In addition, a free carboxyl group appears to be important for inhibition of fermentation, because fatty acid derivatives, such as SCFA Ca salts (JENKINS; PALMQUIST, 1982), alcohols (CZERKAWSKI et al., 1966), amides of fatty acids (FOTOUHI; JENKINS, 1992) and triglycerides (CHALUPA, et al., 1984), inhibit fermentation less.

Combined lipid sources (saturated and unsaturated fatty acids) may enhance fermentation compared to individually provided lipid sources. Jenkins (1987) confirmed that combined fats lower the acetate: propionate ratio, but this effect probably can not be attributed solely to the response of the combination of the fatty acids acting in synergy. Wainman & Dewey (1987) found no evidence of synergism between saturated and unsaturated fatty acids on their energy efficiencies for maintenance and production. However, the effect of lipids on fermentation involves other factors besides the amount and type of dietary fat. Changes in the ruminal concentration of unsaturated fatty acids should be considered because this lipid fraction may generate negative effects on fermentation capacity more than other lipid fractions (saturated fatty acids and triglycerides). The concentration of unsaturated fatty acids in the rumen is regulated by the amount and type of lipid added in the diet, feed frequency, as well as the rates of lipolysis, biohydrogenation, and the formation of carboxylate salts. The high concentrations of triglycerides in the diet increase the total content of lipids in the rumen, however, the increase in rumen unsaturated fatty acids may be lower if lipolysis and biohydrogenation are decreased or if the formation of carboxylate salts is high (HAWKE; SILCOCK, 1970). It should be noted that the degree of carboxylate salt formation depends on the solubility of Ca in diet, dietary lipid content, ruminal pH, fatty acid saturation and chain length (JENKINS; PALMQUIST, 1982).

The mechanism of action of lipids explaining the inhibition of ruminal fermentation is related to the theory of "fiber coating" and the theory of direct antimicrobial effects. Other theories explaining the changes in ruminal fermentation parameters are related to changes in the ruminal population due to decreased cellulose digestion, as well as a reduction in the availability of Ca required for proper microbial function (DEVENDRA; LEWIS, 1974). The fatty acids added to pure cultures of ruminal bacteria inhibit microbial growth, which demonstrates the direct antimicrobial effect of lipids (GALBRAITH et al., 1971; HENDERSON, 1973; MACZULAK et al., 1981). In this case, the lipids in the rumen can inhibit fermentation by partitioning the microbial plasma membrane and then disrupt its function (GRUBER; LOW, 1988). However, if lipids do not interfere with the binding of bacteria to food particles, they may interfere with the binding of bacterial cellulases to cellulose. On the other hand, substitution of a carboxylate group of the fatty acid with another functional group, such as an alcohol or aldehyde, substantially reduces its negative effects on rumen fermentation *in vivo* (CZERKAWSKI et al., 1966) and *in vitro* (JENKINS, 1988). In general, polyunsaturated fatty acids are potential modulators of

rumen fermentation (HARFOOT, 1978; HARFOOT et al., 1974; HENDERSON, 1973; MACZULAK et al., 1981) and the formation of calcium salts of LCFA is a method (JENKINS; PALMQUIST, 1981; PALMQUIST; JENKINS, 1982).

In relation to ruminal parameters, Yang et al. (2009) did not observe ruminal pH changes when the cows were fed with soybean oil and flaxseed, but observed increases in ruminal N-NH₃ concentration. Benchaar et al. (2012) reported no effect on pH, SCFA and N-NH₃ when dairy cows were supplemented with 2%, 3% and 4% dry matter linseed oil. On the other hand, Doreau et al., (2009) with a diet based on silage and addition of linseed oil (3% of DM), observed changes in the concentration of SCFA, while animals fed hay with the same (3%) did not present changes in the concentration of SCFAs (UEDA et al., 2003).

2.3.4.2 Effect of lipids on rumen microorganisms

The results obtained in several studies show that among the main characteristics of lipids that negatively affect the ruminal microbiota are the increase in the degree of unsaturation and the presence of free carboxyl groups (JENKINS, 1993; DOREAU; CHILLIARD, 1997). It has been observed that the negative effect of lipids on digestibility is lower in diets with sufficient amounts of soluble calcium (WHITE et al., 1958) and the productive results are less affected when the diets contain more amount of forage, mainly in the form of hay (SMITH; HARRIS, 1993).

The protective effect of calcium may be due to the blocking of the carboxyl group of free fatty acids, forming calcium salts (PALMQUIST et al., 1986); which facilitates the adhesion of the bacteria to the food particles (ROGER et al., 1990). As for forage, there is the possibility that the particles present in the rumen indirectly protect the bacteria when competing advantageously with them by the adhesion of the fatty acids (HARFOOT et al., 1974).

Palmquist & Jenkins (1980) indicate that the main cause of fiber rumen digestibility depression in response to the inclusion of unprotected lipid sources is probably inhibition of the growth of ruminal microorganisms with fibrolytic activity. The main bacterial species that participate in the ruminal fermentation of the fiber are *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivibrio* and *Prevotella spp.* Protozoa may contribute 5 to 21% of fibrolytic activity (DIJKSTRA;

TAMMINGA, 1995). All entodiniomorph protozoa, with the exception of *Entodinium spp.*, Have cellulases and the highest activity corresponds to *Eudoplodinium maggi*.

In relation to rumen bacteria, different in vitro studies with bacterial cultures have demonstrated a negative effect of fatty acids on their growth. This action is even greater with the addition of polyunsaturated fatty acids (DEMEYER; HENDERICKX, 1967; GALBRAITH et al., 1971; HENDERSON, 1973), with the *cis* form than *trans* (MACZULAK et al., 1981).

As mentioned, polyunsaturated fatty acids (PUFAs) have toxic effects on rumen microorganisms, therefore, lipid supplementation leads to changes in the ruminal microbial population and ruminal fermentation parameters (ZHANG et al., 2008). Due to the toxicity generated by the lipids, mainly of unsaturated type, the bacteria of the rumen, developed a defense mechanism known as biohydrogenation (MAIA et al., 2010). Biohydrogenation is a natural mechanism, performed by the ruminal microorganisms, whose function is to decrease the deleterious effect of lipids, promoting the hydrolysis of esterified lipids, with subsequent hydrogenation of free fatty acids. (HARFOOT; HAZLEWOOD, 1988; JENKINS, 1993). The main responsible for this hydrogenation are the bacteria *Butyrivibrio fibrisolvens*, *Anaerovibrio lipolytica* and *Propionibacter* (BAUMAN et al., 1999; PARIZA et al., 2001).

In relation to protozoa, their role in biohydrogenation is not conclusive. Bonhomme (1990) cites that the ciliates are responsible for 30 to 40% of the lipolytic activity, besides participating in the hydrogenation of fatty acids. Williams & Coleman (1988); Yokoyama & Johnson (1993) affirm that there is evidence of the participation of protozoa in the biohydrogenation of fatty acids. Even though they have lipolytic and hydrogenation activity, protozoa are sensitive to lipids, which is why some oils are used to promote defaunation in ruminants (VAN NEVEL; DEMEYER, 1988; MACHAMÜLLER; KREUZER, 1999).

In a meta-analysis performed by Guyader et al. (2014) report that the anti-protozoal effect of lipids depends on the fatty acid composition of the lipid source, and the medium chain fatty acids are more effective than polyunsaturated fatty acids. Supplements rich in polyunsaturated fatty acids, such as linoleic acid (C18:2 present in soybean oil and sunflower) and linolenic acid (C18:3, present in linseed oil), showed a deleterious effect on methane production of 4.1 and 4.8% in percentage per unit of percentage of added lipid, respectively (MARTIN et al., 2010). Ikwuegbu & Sutton (1982); Towne et al. (1990); Ivan et al. (2001), evaluating the inclusion of lipids in ruminant diets, showed decrease and even

total disappearance of protozoa when the nutrient was added, with *Entodinium* being the least affected. Ikwuegbu & Sutton (1982); Bonhomme (1990) and Ushida et al. (1990) have shown that reduction or elimination of protozoa results in decreased fiber digestion. On the other hand, Czerkawski et al. (1975); Ikwuegbu & Sutton (1982) demonstrated the negative effect of linseed oil on protozoa, and Broudiscou et al. (1994) found that it was also harmful to cellulolytic bacteria and methanogenic *Archaeas*. Studies made (IKWUEGBU; SUTTON, 1982; TOWNE et al., 1990; VAN NEVEL; DEMEYER, 1988; MACHAMÜLLER; KREUZER, 1999 and; IVAN et al., 2001) report the deleterious effect of lipids on ciliate protozoa because of the toxicity generated for the ruminal microbiota when saturated and saturated lipids are added at levels of 3.5 to 6.0% of dry matter in ruminant animals.

Váradyová et al. (2007) indicated that the inclusion of 5% of linseed oil in the sheep diet reduced the total amount of protozoa in relation to the inclusion of sunflower oil, canola and a control diet ($5,0 \times 10^4$ vs $7,5 \times 10^4$, $7,2 \times 10^4$ and; $8,9 \times 10^4$ cells/ml, respectively). On the other hand, Oldick & Firkins (2000) observed that the increase in the degree of unsaturation (from 12,8 to 109,7 iodine value) of the lipid source added in the diet generated a linear reduction in the number of protozoa ($8,7 \times 10^5$ to $5,1 \times 10^5$ cells / ml). Yang et al. (2009) compared the inclusion of soybean oil with linseed oil and a combination of the two in equal amounts (4% DM) in the cow diet and observed a decrease in fibrolytic bacteria and protozoa in animals fed lipids to a control diet ($3,25 \times 10^8$ vs $4,66 \times 10^8$ CFU / mL and $9,04 \times 10^4$ vs $12,92 \times 10^4$ cells / mL, respectively). In a study by Beauchemin et al. (2009) the amount of protozoa in relation to the control diet was significantly reduced in response to the inclusion of 4% extra fat in the form of sunflower and canola, but not flaxseed ($8,28 \times 10^5$ vs $5,16 \times 10^5$, $5,23 \times 10^5$ and $6,35 \times 10^5$ cells / ml, respectively). These studies suggest that the effect of sources of unprotected fat on rumen microorganisms responsible for fiber digestion depends on both the degree of unsaturation and the form of presentation.

On the other hand, the anti-methanogenic effect of polyunsaturated fatty acids has been related to its toxic effect on cellulolytic bacteria (NAGARAJA et al., 1997) and protozoa (DOREAU; FERLAY, 1995). Several studies have reported reduced ruminal methanogenesis by supplementing lauric acid (C12:0) and myristic acid (C14:0), either in pure forms or in products rich in these fatty acids (coconut oil) both in vitro (DOHME et al., 2001; SOLIVA et al., 2004) And *in vivo* conditions (MACHMULLER et al., 2003; JORDAN et al., 2006). Martin et al. (2010) reported that medium chain fatty acids, mainly

provided by coconut oil, resulted in a CH_4 decrease of 7,3% per unit percentage of added lipids. In a study by Faciola & Broderick (2014), the authors observed that coconut oil and lauric acid reduced the number of protozoa by 40%; however, lauric acid altered fiber digestibility, while coconut oil did not. On the other hand, reductions in CH_4 production and a concomitant decrease in dry matter intake were also observed when coconut oil and lauric acid were used as defatting agents (HRISTOV et al., 2013).

2.3.4.3 Lipids as a strategy to mitigate methane emissions

The effectiveness of lipid addition to reduce methane emissions depends on several factors, including the level of supplementation, the lipid source used, the form of delivery (refined oil or oilseed seeds, for example), the type of diet (BEAUCHEMIN et al., 2008) and the degree of fatty acid saturation (FA). On the other hand, the use of lipid supplementation as an enteric methane mitigation strategy should consider the effects on the metabolism and performance of the animal, so that the choice of the source of oil or fat and the level of addition in the diet are appropriately established. The negative effects of the use of lipids for ruminants are less expressive in diets with low fiber level. Therefore, supplementation with lipids should be more cautious in pasture systems (MACHADO et al., 2011).

Different mechanisms of action of the lipids are known that contribute to the reduction of the methanogenesis process, among them, the reduction of the fermentable organic matter in the rumen, since the lipids are not energy source for the ruminal bacteria; the reduction of methanogenic activity by the presence of medium chain fatty acids (MACHMULLER et al., 2003; MACHADO et al., 2011); or the toxic effect on cellulolytic bacteria (NAGARAJA et al., 1997) and protozoa (DOREAU; FERLAY, 1995), exerted by unsaturated fatty acids and biohydrogenation of polyunsaturated fatty acids (JOHNSON; JOHNSON, 1995). production of propionic acid in the rumen. For this latter mechanism, it has been attributed that fatty acids, mainly unsaturated, can serve as electron acceptors during the biohydrogenation process in the rumen (HEGARTY, 1999). This is because long chain fatty acids are non-fermentable and therefore can decrease the percentage of CH_4 that can be produced (JOHNSON; JOHNSON, 1995). In addition, long chain fatty acids generate a toxic effect on the cell membrane, particularly Gram-positive bacteria. Linoleic acid is toxic to cellulolytic bacteria (*F. succinogenes*, *R. albus* and *R. flavefasciens*), as they affect cell integrity (MAIA et al., 2007). Such changes in the

ruminal microbial population favor the formation of propionate, increasing H_2 uptake in this process.

Studies with LCFA added in pure cultures of ruminal microorganisms demonstrated that the methanogenic ones were very sensitive, whereas, in the majority of the cases, the Gram-negative microorganisms, important for the formation of propionate were less inhibited than the Gram-positive species, explaining the typical effect of lipid toxicity on rumen metabolism (HENDERSON, 1973; MACZULAK et al., 1981). This toxic action of LCFA is due to the adsorption on the cell wall, which alters the passage of nutrients (HENDERSON, 1973).

Normally, triglycerides are used as lipid supplements, and their end effect is dependent on the rate of lipolysis. This ratio can be reduced by feeding on high-grain diets that generate low pH values in the rumen (LATHAM et al., 1972; DEMEYER, 1973). Supplementation with lipids often results in much lower numbers of ciliate protozoa in the rumen (CZERKAWSKI et al., 1975; BROUDISCOU et al., 1990). Since some methanogens are associated with ciliates by the transfer of interspecies hydrogen, it can be speculated that the decrease in the number of protozoa after lipid supplementation may inhibit methanogenesis. It is evident that methanogenesis is directly affected by the presence of protozoa in the rumen (NEWBOLD et al., 1995). Due to the symbiotic relationship between methanogens and protozoa (FINLAY et al., 1994) it is possible to indicate that the release of methane can be reduced by 20 to 30% by detonation of protozoa in the rumen (KREUZER et al., 1986).

Because lipids are considered as a nutritional strategy for handling ruminal fermentation and mitigating enteric methane emissions (BOADI et al., 2004), it has been indicated that the addition of 1% of fat in high rations in concentrate decreases production of methane in the rumen in 5.6% (BEAUCHEMIN et al., 2007). However, higher amounts of lipids in the diet (> 5%) decrease the degradation of organic matter and fiber in the rumen (DEMEYER; DEGREAVE, 1991), as well as feed consumption (ALLEN, 2000) therefore, cellulolytic activity in the rumen (PALMQUIST, 1984).

It has been reported that oils such as sunflower and canola, which are rich in long-chain fatty acids, reduce methane emissions in predominantly bulky-fed cattle, thereby increasing gross energy efficiency by up to 22% (BEAUCHEMIN; MCGINN, 2006). In a study carried out in sheep, it was reported that sunflower oil added to the feed (6% in DM) decreased the number of protozoa and, consequently, decreased rumen methane production (IVAN et al., 2001).

2.3.4.4 Monensin effect on ruminal fermentation parameters

The action of the ionophores in the rumen occurs by changes in the microbial population, selecting Gram-negative, succinic acid or lactic acid fermenting bacteria, and inhibiting Gram-positive, acetic, butyric and lactic acid and also H_2 inhibitors (MORAIS et al., 2006). Gram-negative bacteria are more resistant to ionophores because their cell consists of a cell wall and a protective outer membrane that prevents the entry of ionophores. On the other hand, Gram-positive bacteria have only a thick layer of peptidoglycans which, being porous, does not impede the action of monensin. In general, all the effects of ionophores are secondary to the phenomenon caused by the alteration of the normal physiology of the cell membrane of microorganisms (MACHADO et al., 2011). Besides the cell wall is an important barrier against the action of ionophores in Gram-negative bacteria (RUSSEL; STROBEL, 1988), other mechanisms of resistance have already been described. The ionophores exert a selective action on the bacteria, so that the Gram-negative ones survive thanks to the enzymatic system fumarate-reductase most prevalent in this type of microorganism, which couples the electron transport to the extrusion of protons via the plasma membrane (CHEN; WOLIN, 1979).

Among the effects of monensin on ruminal fermentation, studies indicate that this ionophore decreases the production of lactic acid in vitro (DENNIS et al., 1981), as well as in vivo. In cattle supplemented with monensin, a decrease in lactate concentrations has been observed, as well as increased ruminal pH values (NAGARAJA et al., 1982). Other studies show that the total production of short chain fatty acids (SCFA) is not affected, but that there is a decrease in the acetate: proprionate ratio, since propionate producing bacteria and lactate users are favored, and the acetate producers, butyrate, lactate, and ammonia are disadvantaged (RUSSELL; STROBEL, 1989, HRISTOV et al., 2001, GUAN et al., 2006).

The use of ionophores also results in improved ruminant protein utilization. Basically, such benefit is due to the fact that the proteolytic and fermentative bacteria of amino acids are sensitive to ionophores, which decreases the concentration of N-ammoniacal in the ruminal fluid (GOMES, 2009). According to McGuffey et al. (2001) the accumulation of alpha-amino-nitrogen and peptides suggests that monensin generates greater inhibition of the deamination than of the proteolysis itself.

2.3.4.5 Monensin effect on rumen microorganisms

Ionophores are generally bacteriostatic rather than bactericidal (NAGARAJA; TAYLOR, 1987) and their mechanisms of action are their ability to alter the flow of cations through the membrane. Monensin makes the sodium/potassium antigen, decreasing the cellular potassium concentration and the influx of protons, resulting in lower intracellular pH. Since the intracellular pH is low, monensin catalyzes a flow of protons with sodium (RUSSEL, 1987; CHEN; RUSSEL, 1989).

The mechanism of action of ionophores on ruminal bacteria is related to resistance factors present in the cell wall structure, and this is responsible for regulating the chemical balance between the internal and external environment of the cell, this balance being maintained by a mechanism called ionic pump. Ionophores, when they bind to the cell membrane of bacteria and protozoa, as well as, probably to that of rumen fungi, facilitate the movement of cations through the cell membrane into the bacterium, leading to the reduction of the intracellular concentration of K^+ , low pH and higher intracellular concentration of Na^+ (MORAIS et al., 2006). Microorganisms, by means of the ion pump mechanism, in an attempt to maintain their osmolarity, use their energy excessively (they are forced to use cellular transport systems to dissipate H^+ and intracellular Na^+ in an attempt to maintain equilibrium (MORAIS et al., 2006), which affects the growth of gram-positive bacteria (MORAIS et al., 2006) positive and favors the gram-negative (RANGEL et al., 2008). The interaction between ionophore-mediated cation and proton (H^+) begins when the anionic ionophore forms at the membrane contact surface, where it is stabilized by the polar environment (BERGEN; BATES, 1984). As an anion, the ionophore is capable of binding to an ion, a cationic metal; both with carboxylic acid termination. This binding initiates the formation of a complex lipophilic-lipophilic complex (GONÇALVES et al., 2012).

When monensin binds to the cell membrane, the first reaction that occurs is the rapid outflow of K^+ and entry of H^+ into the cell, due to the gradient difference in K^+ concentration. The H^+ accumulated inside the cell causes a reduction of the pH, and the cell responds to the drop in pH with a second reaction, exporting H^+ to the outside environment and allowing Na^+ to enter the cell, cation of greater affinity for the monensin (RUSSEL, 1987). H^+ is exported through Na^+ / K^+ ATPase and / or proton ATPase pumps, using energy in this process (BAGG, 1997). Much of the energy produced by the cell is used by the Na^+ / K^+ and proton ATPase pumps, in an attempt to maintain pH and

cellular ion balance. As a consequence, the ion pump does not operate efficiently, an imbalance is generated due to the higher concentration of cations within the cell and an increase in the osmotic pressure occurs; the water penetrates in excess and thus the cell tends to break (MORAIS et al., 2006), so that, over time, the cell becomes unable to maintain its energy metabolism, decreasing its growth capacity and reproduction, and end up dying or assuming a microbial niche without ruminal expression.

NAGARAJA et al. (1981) evaluated the sensitivity of gram-positive, rumen lactate-producing bacteria (*Butyrvibrio fibrisolvens*, *Eubacterium cellulosolvens*, *E. ruminantium*, *Lachnospira multiparus*, *Lactobacillus ruminis*, *L. vitulinus*, *Ruminococcus albus*, *R. flavefaciens* and *Streptococcus bovis*) and gram-negative, lactate fermenters (*Anaerovibrio*, *Megasphaera*, *Selenomonas*) to the monensin and lasalocid ionophore found that most lactate-producing bacteria were inhibited by both ionophores, unlike lactate users, who were not sensitive. However, in addition to bacteria, other microorganisms such as fungi (ELLIOTT et al., 1987) and protozoa (HABIB; LENG, 1986) are also affected by ionophores. In general, increasing the participation of gram-negative bacteria in the rumen alters the final fermentation products, by increasing the propionate ratio and reducing the proportions of acetate and butyrate (McGUFFEY et al., 2001).

In relation to the effects of monensin on protozoa in the rumen, Thivend & Jouany (1983) showed a toxic effect of ionophore on protozoa. According to Dennis et al. (1986) and Arakaki et al. (2000), the continuous supply of monensin may result in a population selection of resistant protozoa.

On the other hand, ionophores inhibit ruminal methanogenesis, however, their mode of action is not directly with methanogenic *Archaeas*. When hydrogen gas was added to mixed cultures (*in-vitro*) containing monensin, the production of methane (VAN NEVEL; DEMEYER, 1977) was increased. This is explained by the fact that with the inclusion of monensin, hydrogen-producing bacteria and formate are inhibited, while the bacteria producing succinate and propionate are more tolerant (CHEN; WOLIN, 1979), indicating that the decrease in Methane production is due to a decline in the production of hydrogen, the primary substrate of methanogenesis in the rumen.

In relation to ruminal fungi, it has been described that these produce large amounts of hydrogen and have proteolytic action (WALLACE; JOBLIN, 1985). There has been considerable speculation about the importance of fungi in cellulose degradation (MOUNTFORT, 1987). In vitro studies have shown that ruminal fungi are also sensitive to monensin (STEWART et al., 1987), but inhibition in vivo has not yet been confirmed.

2.3.4.6 Monensin as a methane mitigation strategy

The anti-methanogenic effects of ionophores are more related to inhibition of the formation of methane precursors (form and H_2) than a direct effect on the population of methanogens, since these are more resistant to ionophores than the bacteria they produce and provide H_2 (MACHADO et al., 2011). Accordingly, the effects of ionophores on methane reduction are considered indirect by inhibiting the growth of gram-positive bacteria, which have final hydrogen and formate intermediates in the formation of methane in the ruminal environment (RANGEL et al. al., 2008). Thus, the increase in propionate production is accompanied by the reduction in the amount of methane produced by the decrease of its precursors.

In vitro experiments confirmed the indirect effect of monensin on methanogenesis, when a reduction of microorganisms producing CO_2 and H_2 was observed, thus depriving the methane-producing bacteria of its most important substrate (VAN NEVEL; DEMEYER, 1977; CHEN; WOLIN, 1979; HENDERSON et al., 1981). Therefore, as indicated above, the effect of ionophores, such as monensin and lasalocid appear to be due to the transport of ions across the cell membrane and increased osmotic pressure within the cell (RUSSELL; STROBEL, 1989; WALLACE, 1994). Consequently, there is a change in the microbial population, reflected in an increase in resistant or less sensitive species of propionate. However, it should be noted that the extent of inhibition or decrease in methane production by ionophores is related to factors such as the dose administered and the diet provided (bulk / concentrate ratio) (VAN NEVEL; DEMEYER, 1996).

In relation to ciliate protozoa, they are also involved in the production of methane, since they present great potential for hydrogen production, and methane production is modulated mainly by the presence of free carbon dioxide and hydrogen in the ruminal environment (GOMES, 2009). Methanogenic microorganisms can be found either attached to protozoa or in their intracellular phase (CHAGAN; USHIDA, 2004), indicating a possible symbiotic relationship in which they use the hydrogen produced by protozoa to favor the maintenance of an adequate ruminal environment. Habib & Leng (1986) performed in vitro and in vivo experiments in which it was observed that protozoa were sensitive to monensin and lasalocid. However, Guan et al. (2006), demonstrated that this sensitivity can be given, but does not persist in the long term. In this experiment, the short-term and long-term effects of the ionophores on the enteric methane emission for 16 weeks were evaluated for cattle fed diets with high and low concentrate content and with the

addition of monensin during the whole experimental period. In this study, monensin decreased the population of ciliate protozoa initially, but the original population was reestablished after the fourth and sixth week (for high and low concentrate, respectively), as well as methane emission. However, the decrease in the acetate: propionate ratio, as well as the N-ammoniacal concentration, persisted for both high and low concentrate diets. According to the authors, such results suggest that the effects of ionophores are related to populations of ciliate protozoa, and that such populations may adapt to the presence of ionophores.

Ionophores can reduce methane losses by 30% (SCHELLING, 1984). When methanogenesis is decreased, other reduced products (most notably propionate) increase at the expense of acetate (WALLACE et al., 1980). Thus, with the increase of propionate, there is more energy available to the animal for productive purposes (RUSSELL; STROBEL, 1989).

2.3.4.7 Lipids effect on Protein Metabolism

The concentration of ammonia is dependent on the equilibrium between two input sources (N degradability of food and N recycling) and three output (incorporation of N in microorganisms, absorption of N and the exit of N through the hole reticle-omasal).

Literature data indicate that lipid supplementation generally does not result in variation in ammonia concentration in the rumen. Very few experiments show an increase in ammonia concentration (ROBERTSON; HAWKE, 1964).

In general, nitrogen recycling is probably not modified by lipids in most experiments and there is no reason for a variation of amino acid catabolism when fat is added (ELMEDDAH et al., 1994). On the other hand, variations in the concentration of ammonia depend mainly on the ruminal pH, however, some authors indicate that the addition of lipids does not modify the pH of the rumen in most cases, except for KOWALCZYK et al. (1977). Likewise, lipid supplementation does not modify the rate of passage and turnover of ruminal fluid, thus, the flow of ammonia out through the reticulum-omasal orifice is not altered.

2.3.4.8 Monensin effect on protein metabolism

The use of ionophores in the ruminant diet generates an increase in glycogen amino acids in the bloodstream from the small intestine (SCHELLING, 1984). Because they affect the development of some bacteria that promote proteolysis and deamination at the rumen level, and reduce protein degradation in that compartment, allowing post-ruminal digestion. This benefit alleviates the mobilization of body tissues, especially for early lactating cows, which has a high demand for glucose, since this is the precursor of milk lactose, and usually these females are in negative energy balance (GONÇALVES et al., 2012).

Ionophores also cause a decrease in the catalysis of peptides in the rumen, increasing their escape into the small intestine (GOES, 2004) with reduction of N-NH₃ (OSCAR et al., 1987). This result implies advantages in the contribution of amino acids by the animal and can contribute to its performance (GONÇALVES et al., 2012).

2.4 ANAEROBIC BIODIGESTION OF BOVINE RESIDUES

Anaerobic biodigestion is presented in the global context of carbon trading as a clean development mechanism that, in short, means being within a set of alternatives, which aim to assume responsibilities for reducing pollutant emissions and promoting sustainable development, under the Kyoto Protocol in 1997 (MOGAMI, 2005).

Anaerobic biodigestion is one of the processes used to treat residues of organic origin. It consists of a natural microbial process of fermentation, where bacteria multiply and produce between 50 and 80% of methane (CH_4) and 20 to 50% of carbon dioxide (CO_2) and traces of other gases, I sit these gases produced a source of useful and renewable energy (MES et al., 2003). In this process, a liquid effluent and a solid sludge (AXAOPOULOS; PANAGAKIS, 2003) are also produced from the decomposition of organic matter (CRAVEIRO et al., 1982). Biodigestion can be used to treat waste both in solid and liquid form and results in biogas considered as an attractive source of energy (CORBITT, 1990) and biofertilizer, which are variable in their constitutions according mainly to the origin of the substrate, as well as the process management mechanism. It is an efficient process of treating considerable amounts of waste, reducing its polluting power and the health risks arising from them (HILLS, 1980).

The bacteria responsible for the anaerobic digestion are arranged in nature, in lake sediments, sanitary landfills, digestive tract of animals (mainly ruminants) and manures (CRAVEIRO et al., 1982). Anaerobic microbiological decomposition is a process in which microorganisms generate energy and grow by metabolizing organic material in an oxygen-free environment, resulting in the production of methane (CH_4). The anaerobic digestion process can be subdivided into the following four phases, each requiring its own characteristic group of microorganisms (MES et al., 2003):

- Hydrolysis: conversion of non-soluble biopolymers into soluble organic compounds
- Acidogenesis: conversion of organic compounds soluble in short chain fatty acids (SCFA) and CO_2
- Acetogenesis: conversion of short chain fatty acids into acetate and H_2
- Methanogenesis: conversion of acetate and CO_2 plus H_2 to methane gas

2.4.1 Parameters that interfere with the biogas production

There are specific parameters that are important for efficient gas production. Thus, for the anaerobic fermentation process to occur, it is necessary to have favorable conditions for the growth of the biomass of microorganisms (RUIZ, 1992). The environmental factors that influence anaerobic digestion mainly involve temperature, pH, alkalinity, adequate macronutrients (N, P, SO_4^{2-}) and micronutrients (trace metals), adequate metabolic time and a source of carbon (for synthesis and energy). The maximum production occurs when these parameters are chosen from the optimum range (MORAES et al., 2015).

2.4.1.1 Temperature

Temperature is one of the main parameters, and the development of the methanogenic Archaeas and the consequent production of biogas is largely due to the temperature used in the process, and the optimum temperature depends on the group of bacteria with which it intends to work (ARRUDA et al., 2002). The chemical and biochemical reactions are directly influenced by temperature, affecting the process of anaerobic digestion, but sudden temperature variations can lead the digestion process to the imbalance and death of the digestive bacteria (CRAVEIRO et al., 1982).

The fermentation process occurs between 15° to 65° C and is divided into three thermal bands: psychrophilic (below 20° C), mesophilic (30° to 40° C) and thermophilic (50° to 65° C) (RUIZ, 1992). However, Corrales et al. (2015), indicates that the range of the fermentation process can be between 10° to 80° C, dividing this process into five bands, as shown in Table 1, in which we can observe the different facultative anaerobic microorganisms found in each range. Anaerobic digestion can occur at temperatures as low as 0 ° C, but the rate of methane production increases with increasing temperature until reaching a relative maximum of 35 to 37 ° C (LETTINGA; VAN HAANDEL, 1993). In this temperature range, the mesophilic microorganisms are involved. The relationship between the need for energy and biogas yield will further determine the choice of temperature. At higher temperatures, thermophilic bacteria replace mesophilic bacteria and a maximal methanogenic activity occurs at about 55 ° C or higher. However, it should be noted that the biodigestion process becomes more effective in the mesophilic range in which the ideal temperature is around 37° C (CRAVEIRO et al., 1982).

Table 1 - Anaerobic microorganisms found in different temperature ranges.

Temperature Range	Microorganism type	Microorganisms found
10 – 15	Psychophiles	<i>Bacillus spp</i>
20 – 30	Psychotrophs	<i>Clostridium spp</i>
30 – 37	Mesophiles	<i>Clostridium spp</i> <i>Methanococcus spp</i> <i>Methanobacterium spp</i>
42 – 46	Thermotrophs	<i>Methanococcus spp</i> <i>Methanobacterium spp</i>
50 – 80	Thermophiles	<i>Clostridium spp</i> <i>Lactobacillus spp</i> <i>Thermus spp</i> <i>Thermococcus spp</i>

Source: Adapted from CORRALES et al. (2015).

2.4.1.2 pH

Alterations of the pH inside the biodigester can drastically affect the bacteria involved in the process (ARRUDA et al., 2002). The first steps of anaerobic digestion can occur in a wide range of pH values, whereas for the methanogenesis process, and the adequate development of the activities of the microorganisms, a neutral or slightly alkaline pH is necessary (LETTINGA; VAN HAANDEL, 1993). The average pH value should be

between 6,5 and 7,5 (RUIZ, 1992; CORRALES et al., 2015), indicating that pH values outside this range result in a lower methane production rate. A sufficient amount of hydrogen carbonate (often indicated as bicarbonate alkalinity) in the solution is important to maintain the optimum pH range required for methanogenesis (MES et al., 2003).

The biodigester overload can cause an acidification of the medium caused by the bacteria responsible for the degradation of the large polymers, because they have an activity with greater formation of organic acids (RUIZ, 1992). Table 2 shows the pH ranges that may arise during the fermentation process and some anaerobic microorganisms associated with each of these ranges.

Table 2 - Anaerobic microorganisms found in different pH ranges.

Temperature Range	Microorganism type	Microorganisms found
1.1 – 5.5	Acidophiles	<i>Lactobacillus spp</i> <i>Bifidobacterium bifidum</i>
5.5 – 8.0	Neutrophiles	<i>Clostridium perfringes</i> <i>Methanococcus sp</i> <i>Methanobacterium sp</i> <i>Propionibacterium acidipropionici</i>
8.0 – 11.5	Acalonophiles	<i>Clostridium botulinum</i> <i>Clostridium sporogenes</i> <i>Clostridium tetani</i> <i>Fusobacterium spp</i> <i>Micrococcus spp</i>

Source: Adapted from Corrales et al. (2015).

2.4.1.3 Umidade

The amount of water used should be around 90% of the total biomass content. The water content varies according to the differences presented by the raw materials, being important that the water content is within this standard (RUIZ, 1992). The dilution should be around 1:1 to 1:2, that is, an amount of water to the other substrate. Both the excess and the lack of water are damaging to the system, where the lack can cause clogging in the pipeline and the excess can disrupt the hydrolysis process, because a high biomass load is required for it to proceed properly (ARRUDA et al. al., 2002).

2.4.1.4 Concentration of nutrients

The main nutrients are the organic ones, mainly the carbon, the nitrogen and trace elements in very low concentration. There must be a coherent carbon / nitrogen (C/N) ratio according to the biogas production standards, where it must be maintained between 20:1 and 30:1, that is, the carbon concentration of 20 to 1 of nitrogen, where the excess of nitrogen can lead to poor biogas formation, and may have as nitrogenous compounds such as ammonia (ARRUDA et al., 2002).

Phosphate is a necessary element, indicating that its absence can inhibit the process. Sulfur is another basic nutrient for bacteria. However, at very high levels, there will be an excessive growth of sulfate-reducing bacteria, converting the sulfur salts into hydrogen sulfide, a poisonous gas and difficult to burn methane (NOGUEIRA, 1992).

2.4.1.5 Retention time

The retention time may vary from reaction to reaction. It usually takes 30 to 45 days, but in some situations it is possible to have biogas in the first week of hydraulic retention, of course, in smaller proportions. In continuous biodigester systems it is more common to observe this variation (ARRUDA et al., 2002).

In general, the retention time indicates the period in which the organic material remains in the biodigester, until its complete degradation occurs (NOGUEIRA, 1992). The higher the daily loading volume, the shorter the retention time, however the reduced retention time may make the digestion incomplete, triggering an imbalance in the process (CRAVEIRO et al., 1982).

2.4.2 Substrate importance in the biodigestion process

The substrate can be defined as any material that can be decomposed by biological causes, that is, by the action of different types of bacteria. In general, the substrate can be described as the total mass of organic matter that accumulates inside a space (GASPAR, 2003). Within the anaerobic biodigester, as its name implies, anaerobic biodigestion occurs, which is nothing more than a process of degradation, transformation or decomposition of organic matter, having the biogas and biofertilizer as its final product (FONSECA et al., 2009). Thus, it can be used as substrates for this process: human waste,

cattle manure, swine, equine, goat, poultry, domestic sewage, vinasse, herbaceous plants, agricultural rejects and grasses in general (ARRUDA et al., 2002).

According to Amaral et al. (2004), bovine manure is an organic compound with high energy content, with macro and micronutrients that provide water, shelter and temperature, being preferred by numerous micro and macro vectors of great sanitary importance, using it as an ecological niche, fixing the material and producing the fermentation. When they undergo anaerobic treatment in biodigesters, they can have a good reduction in the amount of pathogenic bacteria and intestinal parasites, normally found in these types of waste, and can still be used as fertilizer. However, if they are not handled and used correctly, their degree of contamination may be increased.

When supplying the biodigester, it is important to take the utmost care in mixing the substrate with a compatible volume of water, where the amount of water usually is almost equal to that of dry matter. This correctly applied procedure ensures the normal flow of loading and unloading in the continuous loading digesters as well as the normal production of biogas. The performance of the anaerobic biodigesters is affected primarily by the retention time of the substrate in the reactor and the reduction of contact between the substrate used in the supply and the population of viable bacteria (KARIM et al., 2005). According to Souza (2001), the composition of biogas from animal waste varies according to the nature of the fermented matter and throughout the fermentation process, that is, the decomposition of the organic material is differentiated according to its physical, chemical and biological characteristics resulting from the composition of the diet and the type of waste management.

Another important aspect to be observed in anaerobic biodigestion is the total solids content of the substrate. Lucas Jr. (1998) found better biogas production in batch model biodigesters when the total solids content of the substrate was less than 8%, compared to a total solids content of 16%. Results on the average total solids reduction of 71,3% and 58,1% of volatile solids in biodigesters supplied with goat waste were found by Al-Masri (2001). These results were higher than those found by Amorim (2002), whose reduction in volatile solids ranged from 28,6% to 45%.

According to Steil (2001), the key point at the start of the process is related to a microbial population adapted to the residue in question, which can be supplied by microorganisms present in the residue itself, or can be introduced into the biodigester through the use of inoculum. Chanakya et al. (1997) suggested that the decomposition rate of biomass residues and the formation of a balanced population of the initial microbiota is

crucial for the anaerobic biodigestion process. The rapidity and extent of colonization of methanogenic Archaeas appear to be dependent on the initial composition of the biomass substrate and the rate of decomposition to produce various methanogenic intermediates. In this way, it can be observed that both the composition of the waste and its correct dilution to form the substrate used to supply the biodigestors are of fundamental importance in the biodigestion process (MOGAMI, 2005).

In summary, from the digestion process, Table 3 shows the optimum operating range of the main parameters of this anaerobic process.

Table 3 - Optimum operating range of the main parameters of the anaerobic digestion process.

Parameters	General track	Hydrolysis and acidogenesis	Metanogenesis
pH	6,8 – 7,4	5,2 – 6,3	6,7 – 7,5
Temperature (°C)	Mesophilic: 35 Thermophilic: 55	25 – 35 -	32 – 42 50 - 58
Total SCFA (mg/L acetic acid)	50 – 250	-	-
Acetic acid (mg / L)	<1000	-	-
Propionic acid (mg/L)	<250	-	-
Redox Potential (mV)	-200 a -350	+400 a -300	<-250
Alkalinity (mg/L CaCO ₃)	1500 – 3000	-	-
Relation C/N	-	10 - 45	20 – 30
Relation C:N:P	350:7:1	100:5:1	120:5:1
Hidrogênio (mg/L)	<100	-	-

Source: Adapted from Khanal (2008); Deublein; Steinhauser (2011).

2.4.3 Stages and microorganisms involved in the biogas production process

According to Chernicharo (1997), anaerobic digestion involves complex metabolic processes, which depend on the activity of four groups of microorganisms: hydrolytic fermentative bacteria, acidogenic fermentations, syntrophic (acetogenic) bacteria and methanogenic Archaeas.

The first step in anaerobic digestion is the hydrolysis of long chain polymers, performed by the hydrolytic fermentative bacteria. As bacteria are unable to assimilate particulate organic matter, hydrolysis of complex particulates (polymers) occurs in simpler dissolved materials (smaller molecules). The main compounds to be hydrolyzed are cellulose, proteins and lipids. The hydrolysis of these complex polymers, some of which

are insoluble, is catalyzed by extracellular enzymes which are excreted by fermentative bacteria, such as proteases and lipases (BATSTONE; JENSEN, 2011).

A number of factors influence the substrate hydrolysis rate, such as reactor temperature, substrate composition, particle size, medium pH and even the concentration of volatile organic acids from the hydrolysis (CHERNICHARO, 2006). Hydrolytic fermentative bacteria constitute a great mixture of species, many are strict anaerobes, such as those of the genus *Clostridium*, and some are optional, such as those of the genus *Citrobacter*, *Enterobacter* and *Escherichia* (SILVA, 2014).

In the second stage, the acidogenic fermentative bacteria, also known as acid-forming agents, convert the products generated in the hydrolysis into simple organic substances such as short chain fatty acids, acetic acid, formic, propionic, butyric and lactic acid, as well as ethanol and mineral compounds (AMAYA, et al., 2013). This group of bacteria has a minimum generation time (approximately 30 minutes) and the highest microbial growth rates, ie multiply rapidly in a short time. Thus, the acidogenic step will only be unsatisfactory if the material to be degraded is not properly hydrolyzed. In general, the fermentative population is strictly anaerobic and accounts for about 90% of the total bacterial population of the digesters. However, some species are optional and can metabolize the organic material oxidatively (VAN HAANDEL; LETTINGA, 1994).

The main genera of acidogenic fermentative bacteria are: *Clostridium*, *Bacteroides*, *Ruminococcus*, *Butyribacterium*, *Propionibacterium*, *Eubacterium*, *Lactobacillus*, *Streptococcus*, *Pseudomonas*, *Bacillus*, *Escherichia*, *Desulfobacter* and *Micrococcus*.

In the third phase, called acetogenesis, the acetogenic syntrophic bacteria oxidize organic intermediates, such as propionate and butyrate, in acetate, hydrogen and carbon dioxide, which are substrates for methanogenic microorganisms (CHERNICHARO, 1997; MES et al., 2003). The acetogenesis reaction is shown below (LABIB, et al., 1992):

The acetogenic bacteria responsible for this phase are: *Syntrophobacter wolinii* and *Syntrophomonas wolfei*. Other bacteria are: *Clostridium* spp., *Peptococcus anerebus*, *Lactobacillus* and *Actinomyces* (MOLINO, et al., 2013).

As the final stage of the anaerobic conversion process of organic compounds, there is methanogenesis, a process performed by methanogenic *Archaeas* that convert acetate and hydrogen to methane and carbon dioxide. It should be noted that these microorganisms

are divided into two groups: acetoclastic or acetotrophic methanogenic *Archaeas*, which use acetate as a source of carbon and energy for the production of carbon and methane, accounting for about 60-70% of all production of methane, and hydrogenotrophic methanogenic *Archaeas*, which use carbon as a source of carbon and hydrogen as a source of energy for methane production (CHERNICHARO, 1997), the latter being responsible for 30% of methane from the reduction of CO_2 and H_2 (YANG; GUO, 1990). Methanogenic reactions can be written as:

Methanogenic *Archaeas* are strict anaerobes that include: *Methanobacterium*, *Methanobacillus*, *Methanococcus* and *Methanosarcine* (MOLINO, et al., 2013). The main genera of the acetoclastic methanogenic group are: *Methanosarcinas* and *Methanosaetas*. The most common genotypes of hydrogenotrophic methanogens in anaerobic reactors are: *Methanobacterium*, *Methanospirillum*, *Methanobrevibacter*, *Methanoculleus* and *Methanocorpusculum*.

According to Comastri Filho (1981), of all stages of the biodigestion process, methanogenic is the most sensitive and demanding, requiring several precautions to properly process, thus releasing a good production of biogas. Accordingly, for a stable digestion, it is vital that several biological conversions remain sufficiently coupled during the process, to avoid accumulation of intermediate compounds. For example, an accumulation of short chain fatty acids will result in a decrease in pH, which is a condition where methanogenesis can not occur any more. If hydrogen pressure becomes too high, reduced short chain fatty acids are formed, which again result in a decrease in pH (MES et al., 2003).

REFERENCES

- ALLEN, M.S.; MERTENS, D.R. Evaluating constraints on fiber digestion by rumen microbes, **Journal of Nutrition**, v.118, p.261-270, 1988.
- ALLEN, M.S. Effects of diet on short-term regulation of feed intake by lactating dairy cattle, **Journal of Dairy Science**, v.83, n.7, p.1598-1624, 2000.
- AL-MASRI, M.R. Changes in biogas production due to different ratios of some animal and agricultural wastes, **Bioresource Technology**, v.77, n.1, p.97-100, 2001.
- AMARAL, C.C.; AMARAL, L.A.; JÚNIOR, J.L.; NASCIMENTO, A.A.; FERREIRA, D.S.; MACHADO, M.R.F. Biodigestão anaeróbia de dejetos de bovinos leiteiros submetidos a diferentes tempos de retenção hidráulica, **Revista Ciência Rural**, v.34, n.6, p.1897-1902, 2004.
- AMAYA, O. M.; BARRAGÁN, M. T .C.; TAPIA, F. J. A. Chapter 18-Microbial Biomass in Batch and Continuous System. **Biomass Now – Sustainable Growth and Use**, 2013.
- AMORIM, A.C. **Caracterização dos dejetos de caprinos: reciclagem energética e de nutrientes**, Jaboticabal: Universidade Estadual Paulista, 2002, 108p, Dissertação (Mestrado em Produção Animal) - Universidade Estadual Paulista, 2002.
- ANDRADE, P. Técnica in situ (saco de náilon) na avaliação de alimentos para ruminantes, In: SIMPÓSIO INTERNACIONAL DE PRODUÇÃO DE RUMINANTES, REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, Maringá, **Anais,,** Maringá: Eduem, p. 141-147, 1994.
- ARAKAKI, L.C.; STAHRINGER, R.C.; GARRET, J.E. et al, The effects of feeding monensin and yeast culture, alone or in combination, on the concentration and generic composition of rumen protozoa in steers fed on low-quality pasture supplemented with increasing levels of concentrate, **Animal Feed Science and Technology**, v.84, p.121-127, 2000.
- ARRUDA, M.H.; AMARAL, L.P.; PIRES, O.P.J.; BARUFI, C.R.V. Dimensionamento de Biodigestor para Geração de Energia Alternativa. **Revista Científica Eletrônica de Agronomia da Faculdade de Agronomia e engenharia florestal**, v.1, n.2, 2002.
- AXAOPOULOS, P.; PANAGAKIS, P. Energy and economic analysis of biogas heated livestock buildings. **Biomass and Energy**, v.24, p.239-248, 2003.
- BAGG, R. Mode of action of ionophores in lactating dairy cattle, In: USEFULNESS OF IONOPHORES IN LACTATING DAIRY CATTLE, 1997, Guelph, **Proceedings...** Guelph: Ontario Veterinary College, p. 13-21, 1997.
- BAKER, S.K. Rumen methanogens and inhibition of methanogenesis. **Australian Journal of Agricultural Research**, v.50, n.8, p.1293-1298, 1999.
- BALIEIRO NETO, G.; MELOTTI, L. Efeitos de níveis de sebo sobre a degradabilidade in situ do farelo de soja e do feno de Tifton (*Cynodon dactylon* (L.) Pers.) em vacas secas.

Brazilian Journal of Veterinary Research and Animal Science, v. 44, n.4, p. 243-253. 2007.

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.

BATSTONE, D.J.; JENSEN, P.D. 2011. Treatise on water science. **Wilderer, P.** (ed), **Elsevier, Oxford**, pp. 615-639,

BAUMAN, D. E.; GRIINARI, J, M, Nutritional regulation of milk fat synthesis. **Annual Review of Nutrition**, v, 23, p, 203-227, 2003.

BAUMAN, D.E.; BAUMGARD, L.H.; CORL, B.A. et al, Biosynthesis of conjugated linoleic acid in ruminants. **Proceedings of the American Society of Animal Science, Ruminant Reseach**, v.41, p.215-227, 1999.

BEAUCHEMIN, K.A.; MCGINN S.M. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. **Journal of Animal Science**, v. 84, p.1489-1496, 2006.

BEAUCHEMIN, K.A.; MCGINN, S.M.; BENCHAAAR, 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. 2118-2127, 2009.

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

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

BELANCHE, A.; DE LA FUENTE, G.; MOORBY, J.M., NEWBOLD, C.J. Bacterial protein degradation by different rumen protozoal groups. **Journal of Animal Science**, v. 90, p. 4495–4504, 2012a.

BELANCHE, A.; DE LA FUENTE, G.; PINLOCHE, E.; NEWBOLD, C.J.; BALCELLS J. Effect of diet and absence of protozoa on the rumen microbial community and on the representativeness of bacterial fractions used in the determination of microbial protein synthesis. **Journal of Animal Science**, v. 90, p.3924–3936, 2012b.

BELANCHE, A.; DE LA FUENTE, G.; NEWBOLD C. J. Study of methanogen communities associated with different rumen protozoal populations. **FEMS Microbiology Ecology**, v. 90, p.663–677, 2014.

BELANCHE A.; DE LA FUENTE G.; NEWBOLD C. J. Effect of progressive inoculation of fauna-free sheep with holotrich protozoa and total-fauna on rumen fermentation, microbial diversity and methane emissions. **FEMS Microbiology Ecology**, v.362, p.1–10, 2015.

BENCHAAAR, C.; LETTAT, A.; HASSANAT, F.; YANG, W. Z.; FORSTER, R. J.; PETIT, H. V.; CHOUINARD P. Y. Eugenol for dairy cows fed low or high concentrate diets: Effects on digestion, ruminal fermentation characteristics, rumen microbial populations and milk fatty acid profile. **Animal Feed Science and Technology**, v.178, p. 139-150, 2012.

BERGEN, G.W.; BATES, D.B. Ionophores: their effect on production efficiency and more of action. **Journal of Animal Science**, Champaign, v. 58, n.6, p.1465- 1483, 1984.

BOGGS, D.L.; BERGEN, W.G.; HAWKINS, D.R. Effects of tallow supplementation and protein withdrawal on ruminal fermentation, microbial synthesis and site of digestion, **Journal of Animal Science**, v. 64, p. 970, 1987.

BONHOMME, A. Rumen ciliates: their metabolism and relationships with bacteria and their hosts. **Animal Feed Science and Technology**, v. 30, p. 203-266, 1990.

BOUDON, A.; FAVERDIN, P.; DELAGARDE, R.; LAMBERTON, P.; PEYRAUD, J.L. Effects of rumen or duodenal glucose infusions on intake in dairy cows fed fresh perennial Ryegrass indoors. **Journal of Dairy Science**, v.90, n.9, p.4397-4410, 2007.

BREMMER, D.R.; RUPPERT, L.D.; CLARK, J.H.; DRACKLEY, J.K. Effects of chain length and unsaturation of fatty acid mixtures infused into the abomasum of lactating dairy cows. **Journal of Dairy Science**, v. 81, p.176-188, 1998.

BROUDISCOU, L.; VAN NEVEL, C. J.; DEMEYER, D. L. Effect of soya oil hydrolysate on rumen digestion in defaunated and refaunated sheep. **Animal Feed Science and Technology**, v. 30, n. 1-2, p. 51-67, 1990.

BROUDISCOU, L.; POCHET, S.; PONCET, C. Effect of linseed oil supplementation on feed degradation and microbial synthesis in the rumen of ciliate-free and refaunated sheep. **Animal Feed Science and Technology**, v. 49, p.189-202, 1994.

CHAGAN, I.; USHIDA, K. Detection of methanogens and proteolytic bacteria from a single cell of rumen ciliate protozoa. **Journal of General and Applied Microbiology**, Tokyo, v.50, n.4, p.203- 212, 2004.

CHALUPA, W.; RICKABAUGH, B.; KRONFELD, D.S.; SKLAN, D. Rumen fermentation in vitro as influenced by long chain fatty acids. **Journal of Dairy Science**, v. 67, p. 1439, 1984.

CHANAKYA, H.N.; VENKATSUBRAMANIAM, R.; MODAK, J. Fermentation and methanogenic characteristics of leafy biomass feedstocks in a solid phase biogas fermentor. **Bioresource Technology**, v. 62, p.71-78, 1997.

CHEN, G.; RUSSEL, J.B. More monensinsensitive, ammonia-producing bacteria from the rumen. **Applied and Environmental Microbiology**, Washington, v.55, n.5, p.1052-1057, 1989.

CHEN, M.; WOLIN, M.J. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. **Applied and Environmental Microbiology**, v. 38, p. 72-77, 1979.

CHERNICHARO, C.A.L. **Reatores Anaeróbios - Princípios do tratamento biológico de águas residuárias: biodigestores anaeróbios**, Belo Horizonte: Departamento de Engenharia Sanitária e Ambiental-UFMG, v.5, 2 ed., 1997, 246 p.

CHERNICHARO, C. A. L. Post-treatment options for the anaerobic treatment of domestic wastewater. Reviews in **Environmental Science and Bio/Technology**, v. 5, p.73–92, 2006.

CHILLIARD, Y. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: a review, **Journal of Dairy Science**, v, 76: 3897-3931, 1993.

CHILLIARD, Y.; DOREAU, M.; GAGLIOSTRO, G.; ELMEDDAH, Y. Addition de lipides protégés (encapsulés ou savons de calcium) à la ration de vaches laitières, Effets sur les performances et la composition du lait. **INRA Prod Anim**, v. 6, p. 139-150, 1993.

COLEMAN, G.S. The cellulose content of 15 species of entodiniomorphid protozoa, mixed bacteria and plant debris isolated from the ovine rumen. **Journal of Agricultural Science**, v.104, p.349-360, 1985.

COMASTRI FILHO, J.A. BIOGÁS: Independência energética do pantanal mato-grossense, EMBRAPA, Corumbá-MS, **Circular técnica**, n.9, p.53, 1981.

COOPER, R.; KLOPFENSTEIN T. Effect of Rumensin and feed intake variation on ruminal pH. In: **Scientific Update on Rumensin/Tylan/Mycotil for the Professional Feedlot Consultant**. pp A1–A14. Elanco Animal Health, Indianapolis, IN. 1996.

COPPOCK, C.E.; WILKS, D.L. Supplemental fat in high-energy rations for lactating cows: effect on intake, digestion, milk yield and composition. **Journal of Animal Science**, v. 69, n.9, p. 3826-3837, 1991.

CORBITT, R. A. **Standard Handbook of Environmental Engineering**. 1st edition, New York: McGraw Hill, Inc, 1990.

CORRALES L.C.; ANTOLINEZ, D.M.R.; BOHÓRQUEZ J.A.M.; CORREDOR A.M.V. Bacterias anaerobias: procesos que realizan y contribuyen a la sostenibilidad de la vida en el planeta. **NOVA**, v.13, n. 23, p.55-81, 2015.

CORREA, H.J. Estimación de la degradabilidad efectiva en el rumen mediante métodos numéricos, **Revista Facultad Nacional de Agronomía**, v.61, n.2, p.4654-4657, 2008.

CRAVEIRO, A.M.; LA IGLESIA, M.R. de; HIRATA, Y.S. **Manual de biodigestores rurais**, São Paulo: Ipt, 1982, 61p.

CZERKAWSKI, J.W.; CLAPPERTON, J.L. Fats as energy-yielding compounds in the ruminant diet, Page 249 in Fats in **Animal Nutrition**, J, Wiseman, cd, Butterworths, Boston, MA, 1984.

CZERKAWSKI, J.W.; CHRISTIE, W.W.; BRECKENRIDGE, G.; HUNTER, M.L. Changes in the rumen metabolism of sheep given increasing amounts of linseed oil in their diet. **British Journal of Nutrition**, v. 34, p. 25-44, 1975.

CZERKAWSKI, J.W.; BLAXTER, K.L.; WAINMAN, F.W. The effect of functional groups other than carboxyl on the metabolism of C18 and C12 alkyl compounds by sheep. **British Journal of Nutrition**, v.20, p. 495-508, 1966.

DEMEYER, D.I.; DEGRAEVE, K. Differences in Stoichiometry Between Rumen and Hindgut Fermentation. **Journal of Animal Physiology and Animal Nutrition**, v. 22, p. 50-61, 1991.

DEMEYER, D.I.; HENDERICKX, H.K. The effect of C18 unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. **Biochimica et Biophysica Acta**, v.137, p. 484-497, 1967.

DEMEYER, D.I.; VAN NEVEL, C.J. Effect of defaunation on the metabolism of rumen microorganisms. **British Journal of Nutrition**, v.42, p.515-524, 1979.

DEMEYER, D.I.; FIEDLER, D.; DEGRAEVE, K.G. Attempted induction of reductive acetogenesis into the rumen fermentation in vitro. **Reproduction, Nutrition, Development**, v.36, p.233-240, 1996.

DEMEYER, D. 'Lipidstoffwechsel im Pansen', in: GIESECKE, D, UND HENDERICKX, H, (eds.), **Biologie und Biochemie der Mikrobiellen Verdauung**, BLV Verlagsgesellschaft, Milnchen, pp, 209-234, 1973.

DENNIS, S.M.; NAGARAJA, T.G.; DAYTON, A.D. Effect of lasalocid, monensin and thiopeptin on rumen protozoa. **Research in Veterinary Science**, v.41, p. 251-256, 1986.

DENNIS, S.M.; NAGARAJA, T.G.; BARTLEY, E.E. Effects of lasalocid or monensin on lactate-producing or -using bacteria. **Journal of Animal Science**, v.52, p. 418-426, 1981.

DEUBLEIN, D.; STEINHAUSER, A. Biogas from Waste and Renewable Resources, 2nd. Edition.: Wiley-VCH Verlag GmbH e Co KGaA. Weinheim 2011.

DEVENDRA, C.; LEWIS, D. The interactions between dietary lipids and fibre in sheep. **Animal Production**, v.19, p. 67-76. 1974.

DIAS, A.M.; SILVA, F.F.; VELOSO, C.M.; ÍTAVO, L.C.V.; PIRES, A.J.V.; SOUZA, D.R.; SÁ, J.F.; MENDES, F.B.L.; NUNES NASCIMENTO, P.V. Bagaço de mandioca em dietas de novilhas leiteiras: consumo de nutrientes e desempenho produtivo, **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.60, n.4, p.987-995, 2008.

DIJKSTRA, J.; TAMMINGA, S. Simulation of the effects of diet on the contribution of rumen protozoa to degradation of fibre in the rumen. **British Journal of Nutrition**, v. 74, p. 617-634, 1995.

DOHME, F.; MACHMÜLLER, A.; WASSERFALLEN, A.; KREUZER, M. Ruminant methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. **Letters in Applied Microbiology**, v. 32, p.47-51. 2001.

DOREAU, M.; CHILLIARD, Y. Digestion and metabolism of dietary fat in farm animals, **British Journal of Nutrition**, v. 78, p.S15-S35 1997.

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.

DOREAU, M.; AUROUSSEAU, E.; MARTIN, C. Effects of linseed lipids fed as rolled seeds, extruded seeds or oil on organic matter and crude protein digestion in cows. **Animal Feed Science and Technology**, v.150, p.187-196, 2009.

EADIE, J.M.; GILL, J.C. The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage age-concentrate diet, **British Journal of Nutrition**, v.26, p.155-167, 1971.

EHLE, F.R. Influence of feed particle density on particulate passage from rumen of holstein cow. **Journal of Dairy Science**, v.67, p. 693-697, 1984.

ELLIOTT, R.; ASH, A.J.; CALDERONCORTES, F.; NORTON, B.W.; BAUCHOP, T. The influence of anaerobic fungi on rumen volatile fatty acid concentrations in vivo. **Journal of Agricultural Science, Cambridge**, v.109, n.1, p.13-17, 1987.

ELLIS, W. C.; DELANEY, D. S. Intraruminal responses to monensin in cattle grazing forages, **Beef Cattle Research in Texas**, 1982, Texas Agr, Exp, Sta, p 84, 1982.

ELLIS, W.C.; MATIS, J.H.; POND, K.R.; LASCANO, C.E. TELFORD, J.P. Dietary influences on flow rate and digestive capacity, In: GILCHRIST, F.M.C.; MACKIE, R.I, (Eds.), **Herbivore nutrition in the subtropics and tropics**, Pretoria, South Africa: Science Press, p. 269-293, 1983.

ELMEDDAH, Y.; DOREAU, M.; ROUEL, J.; CHILLIARD, Y. Effects of calcium salt supplementation on dairy cow performances in early lactation, Influence of the nature of concentrates, **Ann, Zootech**, v. 43, p. 341-353, 1994.

EUGÈNE, M.; ARCHIME`DE, H.; SAUVANT, D. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. **Livestock Production Science**, v. 85, p. 81-97, 2004.

FACIOLA, A.P.; BRODERICK, G.A. Effects of feeding lauric acid or coconut oil on ruminal protozoa numbers, fermentation pattern, digestion, omasal nutrient flow and milk production in dairy cows. **Journal of Dairy Science**, v. 97, p.5088-5100, 2014.

FENCHEL T.; FINLAY B.J. The diversity of microbes: resurgence of the phenotype. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 361, p.1965-1973, 2006.

FINLAY, B.J.; ESTEBAN, G.; CLARKE, K.J.; WILLIAMS, A.G.; EMBLEY, T.M.; HIRT, R.P. Some rumen ciliates have endosymbiotic methanogens, **FEMS Microbiology Letters**, v.117, p.157-162, 1994.

FISHER, D.S. A review of a few key factors regulating voluntary feed intake in ruminants, **Crop Science**, v.42, n.5, p.1651-1655, 2002.

FONSECA, F.S.T.; ARAÚJO, A.R.A.; HENDGES, T.L, Analise de viabilidade econômica de biodigestores na atividade suinícola na cidade de Balsas - MA: **um estudo de caso**, In:

CONGRESSO DA SOCIEDADE BRASILEIRA DE ECONOMIA, ADMINISTRAÇÃO E SOCIOLOGIA RURAL, Porto Alegre: Universidade Estadual do Maranhão, Maranhão, 2009.

FORBES, J.M. **Voluntary food intake and diet selection in farm animals**, CAB International, Oxon, UK; 2, 3, 4 and 9 ch, 1995.

FOTOUHI, N.; JENKINS, T.C. Resistance of fatty acyl amides to degradation and hydrogenation by ruminal microorganisms, **Journal of Dairy Science**, v.75, p.1527, 1992.

GALBRAITH, H.; MILLER, T.B.; PATON, A.M.; THOMPSON, J.K. Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. **Journal of Applied Bacteriology**, v.34, p.803, 1971.

GASPAR, R.M.B.L. **Utilização de Biodigestor em Pequenas e Médias Propriedades Rurais com Ênfase na Agregação de Valor: Um Estudo de Caso na Região de Toledo-PR**, 2003, 106 p, Dissertação (Mestrado em Engenharia de Produção) - Faculdade de Engenharia de produção e sistemas, Universidade Federal de Santa Catarina, Florianópolis-SC, 2003.

GOES, R.H.T.B. Aditivos de alimento para bovinos suplementados a pasto, **Cadernos Técnicos de Veterinária e Zootecnia**, Belo Horizonte, v. 43, p. 34- 45, 2004.

GOMES, C.T. **Aditivos (monensina sódica, levedura e probióticos) para bovinos da raça Nelore terminados com rações com concentrado rico em co-produtos**, 2009, 109 p, Dissertação (Mestrado em Ciência Animal e Pastagens) - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, 2009.

GONÇALVES, M. F.; MARTINS, J.M.S.; OLIVEIRA, M.V.; CARVALHO, C.C.M.; ANTUNES, M.M.; FERREIRA, I.C.; et, al, Ionóforos na alimentação de bovinos. **Vet, Not. Uberlândia**, v.18, n. 2, p. 131-146, 2012.

GRAINGER, C.; BEAUCHEMIN, K.A. Can enteric methane emissions from ruminants be lowered without lowering their production? **Animal Feed Science and Technology**, v.166-167, p.308-320, 2011.

GRAINGER, C.; WILLIAMS, R.; ECKARD, R.J.; HANNAH, M.C. A high dose of monensina does not reduce methane emissions of dairy cows offered pasture supplemented with grain, **Journal of Dairy Science**, v.93, p.5300-5308, 2010.

GREEN, B.L.; MCBRIDE, B.W.; SANDALS, D.; LESLIE, K.E.; BAGG, R.; DICK, P. The impact of a monensin controlled-release capsule on subclinical ketosis in the transition dairy cow. **Journal of Dairy Science**, v. 82, p.333-342, 1999.

GRUBER, H.J.; LOW, P.S. Interaction of amphiphiles with integral membrane proteins, I, Structural destabilization of the anion transport protein of the erythrocyte membrane by fatty acids, fatty alcohols, and fatty amines, **Biochim, Biophys, Acta**, 944, p. 414-424, 1988.

GUAN, H.; WITTENBERG, K.M.; OMINSKI, K.H. Efficacy of ionophores in cattle diets for mitigation of enteric methane. **Journal of Animal Science**, v. 84, n.7, p.1896-1906, 2006.

GUYADER, J.; EUGENE, M.; NOZIERE, P.; MORGAVI, D.P.; DOREAU, M.; MARTIN C. Influence of rumen protozoa on methane emission in ruminants: a meta-analysis approach. **Animal**, v. 8, p.1816–1825, 2014.

VAN HAANDEL, A.C.; LETTINGA, G. **Anaerobic sewage treatment: a practical guide for regions with a hot climate**. John Wiley and Sons, 1994. 222 p.

HABIB, G.; LENG, R.A. The effects of monensin on rumen fungi population in sheep given diets based on oaten chaff or wheat straw, In: RECENT ADVANCES IN ANIMAL NUTRITION IN AUSTRALIA, 1986, Armidale, **Proceedings...** Armidale : University of New England, 1986.

HAIMOUD, A.D.; VERNAY, M.; BAYOURTHE, C. et al. Avoparcin and monensin effects on the digestion of nutrients in dairy cows fed a mixed diet. **Canadian Journal of Animal Science**, v.75, n.3, p.379-385, 1995.

HALL, M.B. Isotrichid protozoa influence conversion of glucose to glycogen and other microbial products. **Journal of Dairy Science**, v. 94, p.4589–4602. 2011.

HARFOOT, C. G.; HAZLEWOOD, G. P. Lipid metabolism in the rumen, Page 285 in **The Rumen Microbial Ecosystem**, P, N, Hobson, ed, Elsevier Appl, Sci, Publ, Co., Inc., New York, NY, 1988.

HARFOOT, C. G.; CROUCHMAN, M.L.; NOBLE, R.C.; MOORE, J.H. Competition between food particles and rumen bacteria in the uptake of long chain fatty acids and triglycerides. **Journal of Applied Bacteriology**, v.37, p.663, 1974.

HARFOOT, C. G. Lipid metabolism in the rumen, **Progress in Lipid Research**, v.17, p.21, 1978.

HARRISON, D.G.; McALLAN, A.B. Factors affecting microbial growth yields in the reticulo-rumen, In: RUCKEBUSH, Y.; THIVEND, P, (Eds.), **Digestive Physiology and Metabolism in Ruminants**, Lancaster, England: MTP Press, p.205-226, 1980.

HARVATINE, K.J.; ALLEN, M.S. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. **Journal of Dairy Science**, v.88, p.4018-4027, 2005.

HAWKE, J.C.; SILCOCK, W.R. The in vitro rates of lipolysis and biohydrogenation in rumen contents. **Biochimica et Biophysica Acta**, 218, p. 201, 1970.

HEGARTY, R.S. Reducing rumen methane emissions through elimination of rumen protozoa. **Australian Journal of Agricultural Research**, v.50, p.1321-1327, 1999.

HEGARTY, R.S.; GERDES, R. Hydrogen production and transfer in the rumen, **Recent Advances in Animal Nutrition**, v.12, p.37-44, 1998.

HENDERSON, C.; STEWART, C.S.; NEKREP, E.V. The Effect of Monensin on Pure and Mixed Cultures of Rumen Bacteria. **Journal of Applied Bacteriology**, v.51, p.159-169, 1981.

HENDERSON, C. The effects of fatty acids on pure cultures of rumen bacteria, **Journal of Agricultural Science**, v.81, p.107, 1973.

HILLS, D.J. Methane gas production from dairy manure at high solids concentration, **Transactions of the ASAE**, v.23, n.1, p.122-126, 1980.

HOBSON, P.N.; STEWART, C.S. **The rumen microbial ecosystem**, London: Blackie Academic and Professional, p. 340, 1997.

HRISTOV, A.N.; IVAN, M.; RODE, L.M.; McALLISTER, T.A. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium or high-concentrate barley-based diets. **Journal of Animal Science**, v.79, n.2, p.515-524, 2001.

HRISTOV A. N.; OH, J.; FIRKINS, J.L.; DIJKSTRA, J.; KEBREAB, E.; WAGHORN G.; et al. Mitigation of methane and nitrous oxide emissions from animal operations: a review of enteric methane mitigation options. **Journal of Animal Science**, v. 91, p.5045–5069, 2013.

HUHTANEN, P.; AHVENJÄRVI, S.; WEISBJERG, M.R.; NØRGAARD, P. Digestion and passage of fibre in ruminants, In: SEJRSEN, K.; HVELPLUND, T.; NIELSEN, M.O. (Eds.), **Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress**, Wageningen, the Netherlands: Wageningen Press,, p.87–135, 2006.

HUNGATE, R.E.; SMITH, W.; BAUCHOP, T.; YU, I.; RABINOWITZ, J.C. Formate as an intermediate in the bovine rumen fermentation. **Journal of Bacteriology**, v.102, n.2, p.389-397, 1970.

HUNGATE, R.E. Hydrogen as an intermediate in the rumen fermentation. **Archives of Microbiology**, v.59, p.158–164. 1967.

IKWUEGBU, O.A.; SUTTON, J.D. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. **British Journal of Nutrition**, v.48, p. 365, 1982.

IPHARRAGUERRE, I.R.; CLARK, J.H. Usefulness of ionophores for lactating cows: a review. **Animal Feed Science and Technology**, v.106, p.39-57, 2003.

IRBIS, C.; Ushida, K. Detection of methanogens and proteobacteria from a single cell of rumen ciliate protozoa. **The Journal of General and Applied Microbiology**, v.50, p.203-212, 2004.

ITABASHI, H.; KOBAYASHI, T.; MATSUMOTO, M. The effect of rumen ciliate protozoa on energy metabolism and some constituents in rumen fluid and blood plasma of goats, **Japanese Journal of Zootechnical Science**, v.55, p.248-255, 1984.

IVAN, M.; NEILL, L.; ENTZ, T. Ruminal fermentation and duodenal flow following progressive inoculations of fauna-free wethers with major individual species of ciliate protozoa or total fauna. **Journal of Animal Science**, v.78, p.750–759, 2000.

IVAN, M.; MIR, P.S.; KOENIG, K.M. et al, Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. **Small Ruminant Research**, v.41, p.215–227, 2001.

IVAN, M. Comparison of duodenal flow and digestibility in fauna-free sheep inoculated with Holotrich protozoa, Entodinium monofauna or total mixed protozoa population. **British Journal of Nutrition**, v.101, p.34–40, 2009.

IWAMOTO, M.; ASANUMA, N.; HINO, T. Effect of nitrate combined with fumarate on methanogenesis, fermentation, and cellulose digestion by mixed ruminal microbes in vitro, **Animal Science Journal**, v.70, n.6, p.471–478, 1999.

JACOB, A.B.; BALAKRISHNAN, V.; KATHIRVELAN, C. Effect of amount and source of vegetable oils in a high fibrous cattle diet on in vitro rumen fermentation, nutrient degradability and rumen cis-9, trans-11 CLA concentration. **Journal of Applied Animal Research**, v.40, p.148–153, 2012.

JALC, D.; CERESNAKOVA, Z. Effect of plant oils and aspartate on rumen fermentation in vitro. **Journal of Animal Physiology and Animal Nutrition**, v. 85, p.378–384. 2001.

JARVIS, G.N.; STRÖMPL, C.; BURGESS, D.M.; SKILLMAN, L.C.; MOORE, E.R.B.; JOBLIN, K.N. Isolation and identification of ruminal methanogens from grazing cattle. **Current Microbiology**, v. 40, p. 327–32. 2000.

JENKINS, T.C. Effect of fats and fatty acid combinations on ruminal fermentation in semicontinuous in vitro cultures, **Journal of Animal Science**, v.64, p.1526, 1987.

JENKINS, T.C.; McGUIRE, M.A. Major advances in nutrition: impact on milk composition. **Journal of Dairy Science**, v.89, p.1302–1310, 2006.

JENKINS, T.C.; PALMQUIST, D.L. Effects of three forms of tallow on nutrient digestibility in lactating cows, **Journal of Dairy Science**, v. 64, Suppl, 1, p.129, 1981.

JENKINS, T.C.; PALMQUIST, D.L. Effect of added fat and calcium on in vitro formation of insoluble fatty acid soaps and cell wall digestibility. **Journal of Animal Science**, v.55, p. 957, 1982.

JENKINS, T.C.; PALMQUIST, D.L. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations, **Journal of Dairy Science**, v.67, p.978, 1984.

JENKINS, T.C. Fat interactions in ruminant diets, Page 117, in **Proc, 49th Minnesota Nutr, Conf.**, Univ, Minnesota, Bloomington, 1988.

JENKINS, T.C. Lipid metabolism in the rumen, **Journal of Dairy Science**, v.76, p.3851–3863, 1993.

JOBLIN, K.N. Ruminal acetogens and their potential to lower ruminant methane emissions. **Australian Journal Agricultural Research**, v.50, n.8, p.1321-1327, 1999.

JOHNSON, K.A.; JOHNSON, D.E. Methane Emissions from Cattle, **Journal Animal Science**, v.73, p.2483-2492, 1995.

JONES, W.J.; NAGLE, D.P.; WHITMAN, W.P. Methanogens and the diversity of archaeobacteria, **Microbiological Reviews**, v.53, p.135–177, 1987.

JONES, W.J. Diversity and physiology of methanogens, In: ROGER J.E.; WHITMAN, W.B, (Eds.), **Microbial production and consumption of greenhouse gases: Methane, nitrous oxides and halomethane**, New York: Academic Press Inc., p.39-54, 1991.

JORDAN, E.; LOVETT, D. K.; MONAHAN, F. J.; CALLAN, J.; FLYNN, B.; O'MARA, F. P. Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. **Journal of Animal Science**, v.84, p.162–170. 2006.

JOUANY, J.P.; SENAUD, J. Influence des ciliés du rumen sur la digestion de differents glucides chez ie mouton, I, Utilisation des glucides patietaux (cellulose et hemicelluloses) et de l'amidon, **Reproduction Nutrition Development**, v.22, p.735-752, 1982.

JOUANY, J.P.; ZAINAB, B.; SENAUD, J.; GROLIERE, C.A.; GRAIN J.; TBIVEND, P. Rôle of the rumen ciliate protozoa Polyplastron multivesiculatum, entodininm sp, and Isotricha prostoma in the digestion of a mixed diet in sheep, **Reproduction Nutrition Development**, v.21, p.871-884, 1981.

KAMRA, D.N. Rumen microbial ecosystem, **Current Science**, v.89, p.124-134, 2005.

KARIM, K.; HOFFMANN, R.; KLASSON, K.T.; AL-DAHMAN, M.H. Anaerobic digestion of animal waste: effect of mode of mixing, **Water Research**, v.39, n.15, p.3597–3606, 2005.

KHANAL S.K. **Environmental Factors**. In: Anaerobic Biotechnology for Bioenergy Production: Principles and Applications. S.K Khanal (Ed.). John Wiley & Sons, Inc. USA. pp. 43-63. 2008.

KIM, B.H.; GADD, G.M. **Bacterial physiology and metabolism**, 1a ed, Cambridge: Cambridge University Press; 2008.

KNAPP, J.R.; LAUR, G.L.; VADAS, P.A.; WEISS, W.P.; TRICARICO, J.M. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. **Journal of Dairy Science**, v.97, n.6, 3231-3261, 2014.

KOWALCZYK, J.; GRSKOV, E.R.; ROBINSON, J.J.; STEWART, C.S. Effect of fat supplementation on voluntary food intake and rumen metabolism in sheep. **British Journal of Nutrition**, v.37, p. 251-257, 1977.

KRAJCARSKI-HUNT, H.; PLAIZIR, J.C.; WALTON, J.P.; SPRATT, R.; MCBRIDE, B.W. Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows **Journal of Dairy Science**, v. 85, p.570-573. 2002.

KREUZER, M.; KIRCHGESSNER, M.; MFILLER, H. L. Effect of Defaunation on the Loss of Energy in Wethers Fed Different Quantities of Cellulose and Normal or Steamflaked Maize Starch, **Animal Feed Science and Technology**, v.16, p.233-241, 1986.

KRUMHOLZ, L.R.; FORSBERG, C.W.; VEIRA, D.M. Association of methanogenic bacteria with rumen protozoa. **Canadian Journal of Microbiology**, v.29, p.676-680, 1983.

KUOPPALA, K.; RINNE, M.; AHVENJÄRVI, S.; NOUSIAINEN, J.; HUHTANEN, P. The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows, **Journal of Dairy Science**, v.93, p.3253–3263, 2010.

KUOPPALA, K.; AHVENJÄRVI, S.; RINNE, M.; VANHATALO, A. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows, 2, Dry matter intake and cell wall digestion kinetics. **Journal of Dairy Science**, v.92, p.5634–5644, 2009.

KURIHARA, Y.; EADIE, J.M.; HOBSON, P.N.; MANN, S.O. Relationship between bacteria and ciliate protozoa in the sheep rumen. **Journal of General Microbiology**, v.51, p.267-288, 1968.

LABIB, F.; FERGUSON, J.F.; BENJAMIN, M.M.; MERIGH, M.; RICKER, N.L. Anaerobic butyrate degradation in a fluidized-bed reactor: Effects of increased concentrations of H₂, and acetate. **Environmental Science & Technology**, v. 26, p.369–376, 1992.

LATHAM, M.J.; STONY, J.E.; SHARPE, M.E. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. **Applied Microbiology**, v.24, p.871, 1972.

LAUBE, V.M.; MARTIN, S.M. Conversion of cellulose to methane and carbon dioxide by triculture of *Acetivibrio cellulolyticus*, *Desulfovibrio* sp., and *Methanosarcina barkeri*. **Applied and Environmental Microbiology**, v.42, n.3, p.413-420, 1981.

LEAHY, S.C.; KELLY, W.J.; ALTERMANN, E.; RONIMUS, R.S.; YEOMAN, C.J.; PACHECO, D.M.; LI, D.; KONG, Z.H.; MCTAVISH, S.; SANG, C.; LAMBIE, S.C.; JANSSEN, P.H.; DEY, D.; ATTWOOD, G.T. The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. **PLoS One**, v.5, p.1-17, 2010.

LEITE, R.F. **Ionóforos na digestibilidade e balanço de nitrogênio em ovinos**, 2007, 34f, Monografia (Graduação em Zootecnia) – Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, 2007.

LEMENAGER, R.P.; OWENS, F.N.; SHOCKEY, B.J.; LUSBY, K.S.; TOTUSEK, R. Monensin effects on rumen turnover rate, twenty-four hour VFA pattern, nitrogen components and cellulose disappearance, **Journal of Animal Science**, v.47, p.255, 1978.

LENG, R.A.; NOLAN, J.V. Nitrogen metabolism in the rumen, **Journal of Dairy Science**, v.67, n.5, p.1072-1089, 1984.

LETTINGA, G.; VAN HAANDEL, A. **Anaerobic digestion for energy production and environmental protection**. In: Renewable Energy. Sources for Fuels and Electricity.

Johansson, T.; Kelly, H.; Reddy, A.; Williams, R. (Editors). Earthscan, Island Press. Washington, D.C., US. p. 817-841. 1993.

LITHERLAND, N.B.; THIRE, S.; BEAULIEU, A.D.; REYNOLDS, C.K.; BENSON, J.A.; DRACKLEY, J.K. Dry matter intake is decreased more by abomasal infusion of unsaturated free fatty acids than by unsaturated triglycerides, **Journal of Dairy Science**, v.88, p.632-643, 2005.

LIU, Y.C.; WHITMAN, W.B. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. **Annals of the New York Academy of Sciences** v.1125, p.171-189, 2008.

LUCAS Jr. J. **Aproveitamento energético de resíduos da suinocultura**, In: ENERGIA, AUTOMAÇÃO E INSTRUMENTAÇÃO, Lavras: UFLA/SBEA, p.81-87, 1998.

LUND, P. **The effect of forage type on passage kinetics and digestibility of fibre in dairy cows**, PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 2002.

MACHADO, F.S.; PEREIRA, L.G.R.; GUIMARÃES, R.JR.; LOPES, F.C.F.; CHAVES, A.V.; CAMPOS M.M.; MORENZ, M.J.F. Emissões de metano na pecuária: conceitos, métodos de avaliação e estratégias de mitigação / Fernanda Samarini Machado. [et al.], – Juiz de Fora: **Embrapa Gado de Leite**, 2011, 92p, (Embrapa Gado de Leite, Documentos, 147).

MACHMULLER, A.; KREUZER, M. Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. **Canadian Journal of Animal Science**, v. 79, p.65-72, 1999.

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

MACKIE, R.I.; GILCHRIST, F.M.C.; ROBERTS, A.M.; HANNAH, P.E.; SCHWARTZ, H.M. Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. **Journal of Agricultural Science**, v.90, p.241-254, 1978.

MACZULAK, A.; DEHORITY, E.B.A.; PALMQUIST, D.L. Effects of long-chain fatty acids on growth of rumen bacteria. **Applied and Environmental Microbiology**, v.42, p.856, 1981.

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

MAIA, M.R.G.; CHAUDHARY, L.C.; BESTWICK, C.S.; RICHARDSON, A.J.; McKAIN, N.; LARSON, T.R.; GRAHAM, I.A.; WALLACE, R.J. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. **BMC Microbiology**, v.10, p.52-62, 2010.

MARTIN, C.; MORGAVI, D.P.; DOREAU, M. Methane mitigation in ruminants: from microbe to the farm scale. **Animal**, v.4, p.351-365. 2010.

McALLISTER, T.A. OKINE, E.K.; MATHISON, G.W.; CHENG, K.J. Dietary, environmental and microbiological aspects of methane production in ruminants. **Canadian Journal of Animal Science**, v.76, p.231-243, 1996.

McCARTHY, R.D.; KLUSMEYER, T.H.; VICINI, J.L.; CLARK, J.H.; NELSON, D.R. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. **Journal of Dairy Science**, v.72, p.2002–2016, 1989.

McGUFFEY, R.K.; RICHARDSON, L.F.; WILKINSON, J.I.D. Ionophores for dairy cattle: Current status and future outlook. **Journal of Dairy Science**, v.84, suppl. p.194-203, 2001.

MERTENS, D.R. Predicting intake and digestibility using mathematical-models of ruminal function. **Journal of Animal Science**, v.64, n.5, p.1548-1558, 1987.

MESSANA, J.D. et al . Effects of different lipid levels on protozoa population, microbial protein synthesis and rumen degradability in cattle. **Acta Scientiarum. Animal Sciences**, v. 34, n. 3, p.279-285, 2012 .

MES, T.Z.D. de; STAMS, A.J.M.; ZEEMAN, G. (2003): Chapter 4. **Methane production by anaerobic digestion of wastewater and solid wastes**. In: REITH, J.H. (Editor); WIJFFELS, R.H. (Editor); BARTEN, H. (Editor) (2003): Biomethane and Biohydrogen. Status and perspectives of biological methane and hydrogen production, p. 58-95.

MESSANA, J.D.; BERCHIELLI, T.T.; ARCURI, P.B.; REIS, R.A.; CANESIN, R.C.; RIBEIRO, A.F.; et, al, Rumen fermentation and rumen microbes in Nelore steers receiving diets with different lipid contents. **Revista Brasileira de Zootecnia**, v.42, n.3, p.204-212, 2013.

MOGAMI, C, A, **Influência de diferentes dietas nas características dos dejetos de cabras leiteiras com vistas à produção de biogás**, 2005, 63 p, Dissertação (Magister Scientiae) - Universidade Federal de Viçosa, Minas Gerais - Viçosa, 2005.

MOLINO, A.; NANNA, F.; DING, Y.; BIKSON, B.; BRACCIO, G. Biomethane production by anaerobic digestion of organic waste. **Fuel**, v.103, p.1003-1009, 2013.

MORAES, B.; ZAIAT, M.; BONOMI, A. Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: Challenges and perspectives. **Renewable and Sustainable Energy Reviews**, v. 44, p.888-903, 2015.

MORAIS, J.A.S.; BERCHIELLI, T.T.; REIS, R.A. Aditivos, In: BERCHIELLI, T.T.; PIRES, A.V.; OLIVEIRA, S.G. **Nutrição de ruminantes**, Jaboticabal: Funep, p.111-140, 2006.

MORGAVI, D.P.; FORANO, E.; MARTIN, C.; NEWBOLD, C.J. Microbial ecosystem and methanogenesis in ruminants. **Animal**.v.4, p.1024-1036. 2010.

MORRIS, F.E.; BRANINE, M.E.; GALYEAN, M.L.; HUBBERT, M.E.; FREEMAN, A.S.; LOFGREEN, G.P. Effect of rotating monensin plus tylosin and lasalocid on performance, ruminal fermentation, and site and extent of digestion in feedlot cattle, **Journal of Animal Science**, v.68, p.3069–3078, 1990.

MOSS, A.R.; JOUANY, J.P.; NEWBOLD, J. Methane production by ruminants: its contribution to global warming. **Annales de Zootechnie**, v.49, n.3, p.231-254, 2000.

MOUNTFORT, D.O. The rumen anaerobic fungi, **FEMS Microbiol, Rev**, v.46, p.401-408, 1987.

MÜLLER, M. Review Article: The hydrogenosome, **Journal of General Microbiology**, v.139, n.12, p.2879-2889, 1993.

MUNTIFERING, R.B.; THEURER, B.; NOON, T.H. Effects of monensin on site and extent of whole corn digestion and bacterial protein synthesis in beef steers. **Journal of Animal Science**, v.53, p.1565-1573. 1981.

MUTSVANGWA, T.; WALTON, J.P.; PLAIZIER, J.C.; DUFFIELD, T.F.; BAGG, R.; DICK, P.; VESSIE, G.; MCBRIDE, B.W. Effects of a monensin controlled-release capsule or premix on attenuation of sub-acute ruminal acidosis in dairy cows. **Journal of Dairy Science**, v.85, p. 3454-3461, 2002.

NAGARAJA, T.G.; TAYLOR, M.B. Susceptibility and resistance of ruminal bacteria to antimicrobial feed additives, **Applied and Environmental Microbiology**, Washington, v.53, n.7, p.1620-1625, 1987.

NAGARAJA, T.G.; AVERY, T.B.; BARTLEY, E.E.; GALITZER, S.J. DAYTON, A.D. Prevention of lactic acidosis in cattle by lasalocid or monensin. **Journal of Animal Science, Champaign**, v.53, n.1, p.206-216, 1981.

NAGARAJA, T.G.; AVERY, T.B.; BARTLEY, E.E.; ROOF, S.K.; DAYTON, A.D. Effect of lasalocid, monensin, or thiopeptin on lactic acidosis in cattle, **Journal of Animal Science**, v.54, p. 649-658, 1982.

NAGARAJA, 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, p.523-632, 1997.

NEWBOLD, C.J.; LASSALAS, B.; JOUANY, J.P. The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. **Letters in Applied Microbiology**, v.21, n.4, p.230-234, 1995.

NEWBOLD, C.J.; DE LA FUENTE, G.; BELANCHE, A.; RAMOS-MORALES, E.; MCEWAN, N.R. The role of ciliate protozoa in the rumen. **Frontiers in Microbiology**, v.26, p. 6-13, 2015.

NEWBOLD, C.J.; CHAMBERLAIN, D.G.; WILLIAMS A.G. The effects of defaunation on the metabolism of lactic acid in the rumen. **Journal of the Science of Food and Agriculture**, v.37, p.1083–1090. 1986.

NICHOLSON, T.; OMER, S.A. The inhibitory effect of intestinal infusions of unsaturated long-chain fatty acids on forestomach motility of sheep, **British Journal of Nutrition**, v. 50, p.141–149, 1983.

NOGUEIRA, L.A.H. **Biodigestão: A alternativa energética**, São Paulo: Nobel, p.93, 1992.

NRC. NATIONAL RESEARCH COUNCIL, **Nutrient requirements of dairy cattle**, 7th ed, Washington: National Academies Press, 2001, 381p.

OLDICK, B.S.; FIRKINS, J.L. Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. **Journal of Animal Science**, v. 78, p. 2412-2420, 2000.

ØRSKOV, E.R.; McDONALD, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. **Journal Agricultural Science**, v.92, n.1, p.499-503, 1979.

OSBORNE, J. K.; MUTSVANGWA, T.; ALZAHAL, O.; DUFFIELD, T.F.; BAGG, R.; DICK, P.; VESSIE, G.; MCBRIDE, B.W. Effects of monensin on ruminal forage degradability and total tract diet digestibility in lactating dairy cows during grain-induced subacute ruminal acidosis. **Journal of Dairy Science**, v.87, p.1840-1847, 2004.

OSCAR, T.P.; SPEARS, J.W.; SHIH, J.C. Performance, methanogenesis and nitrogen metabolism of finishing steers fed monensin and nickel, **Journal of Animal Science**, Champaign, v.64, n.3, p.887- 896, 1987.

OWENS, F.N.; GOETSCH, A.L. Ruminal fermentation, In: CHURCH, D.C, (Ed.). **The ruminant animal digestive physiology and metabolism**, New Jersey: Prentice Hall, p.145-171, 1988.

OWEN, J. E.; NORMAN, G. A.; PHILBROOKS, C. A.; JONES, N. S. D. Studies on the meat production characteristics of Botswana goats and sheep-Part 3: Carcass tissue composition and distribution. **Meat Science – Journal**, v.2, p.59-74, 1978.

OWENS, F.N. Ionophore effect on utilization and metabolism of nutrients-ruminants, In: 1980 **Georgia Nutr, Conf**, Univ, of Georgia, Athens, p, 17, 1980.

OXFORD, A.E. The rumen ciliate protozoa: Their chemical composition, metabolism, requirements for maintenance and culture, and physiological significance for the host. **Experimental Parasitology**, v.4, p.569-605, 1955.

PALMQUIST, D.L.; JENKINS, T.C. Fat in lactation rations: review, **Journal of Dairy Science**, v. 63, p.1, 1980.

PALMQUIST, D.L.; JENKINS, T.C. Calcium soaps as a fat supplement in dairy cattle feeding, in **Proc, XIIth World Congr, Dis, Cattle**, Amsterdam, p, 477, 1982.

PALMQUIST, D.L.; JENKINS, T.C.; JOYNER, Jr. A.E. Effect of dietary fat and calcium source on insoluble soap formation in the rumen. **Journal of Dairy Science**, v.69, p. 1020, 1986.

PALMQUIST, D.L.; WEISBJERG, M.R.; HVELPLUND, T. Ruminant, intestinal, and total digestibilities of nutrients in cow fed diets high in fat and undegradable protein, **Journal of Dairy Science**, v.76, p.1353-1364, 1993.

PALMQUIST, D.L. Use of fats in diets for lactating cows, **Proc. Univ. Nottingham Conf, Feed Manuf**, (in press), 1984.

PALMQUIST, D.L. The role of dietary fats in efficiency of ruminants, Conference: regulating lipids metabolism to increase productive efficiency. **Journal of Nutrition**, v.124, p.1377-1382, 1994.

PANTOJA, J.; FIRKINS, J.L.; EASTRIDGE, M.L.; HULL, B.L. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. **Journal of Dairy Science**, v.77, p.2341-2356, 1994.

PARIZA, M.W.; PARK, Y.; COOK, M.E. The biologically active isomers of conjugated linoleic acid, **Progress in Lipid Research**, v.40, p.283-298, 2001.

PAUL, R.G.; WILLIAMS, A.G.; BUTLER, R.D. Hydrogenosomes in the rumen entodiniomorphid ciliate *Polyplastron multivesiculatum*. **Journal of General Microbiology**, v.136, p.388-396, 1990.

PEI, C.X.; MAO, S.Y.; CHENG, Y.F.; ZHU, W.Y. Diversity, abundance and novel 16S rRNA gene sequences of methanogens in rumen liquid, solid and epithelium fractions of Jinnan cattle. **Animal**, v.4, p.20-29, 2010.

POND, K.P.; ELLIS, W.C. Effects of monensina on fecal output and voluntary intake of grazed coastal Bermudagrass, Beef Cattle Research in Texas, 1981, **Texas Agr, Exp, Sta**, p.31, 1981.

PRIMAVESI, O.; FRIGHETTO, R.T.S.; PEDREIRA M.S. Metano entérico de bovinos leiteiros em condições tropicais brasileiras, **Pesquisa Agropecuária Brasileira**, Brasília. v.39, n.3, p.277-283, 2004.

RANGEL, A.H.N.; LEONEL, F.P.; SIMPLÍCIO, A.A.; MENDONÇA JÚNIOR, A.G. Utilização de ionóforos na produção de ruminantes, **Revista de Biologia e Ciências da Terra**, Campina Grande, v.8, n.2, 2008.

ROBERTSON, J.A.; HAWKE, J.C. Studies on rumen metabolism, I, Effects of lipids on the concentration of ammonia, total and individual volatile fatty acids in the rumen, In vivo hydrolysis and hydrogenation of lipids. **Journal of the Science of Food and Agriculture**, v.15, p. 274-289, 1964.

RODRIGUE, C.B.; ALLEN, N.N. The effect of fine grinding of hay on ration digestibility, rate of passage, and fat content of milk. **Canadian Journal of Animal Science**, v.40, p.23-29, 1960.

ROGER, V.; FONTY, G.; KOMISARCZUK-BONY, S.; GOUET, P. Effects of physicochemical factors on the adhesion to cellulose Avicel of the ruminal bacteria *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* subsp. *succinogenes*. **Applied and Environmental Microbiology**, v. 56, p. 3081-3087, 1990.

ROGERS, M.; JOUNANY, J.P.; THIVEND, P.; FONTENOT, J.P. Comparative effects of feeding and duodenal infusion of monensin on digestion in sheep. **Canadian Journal of Animal Science**, v.71, p.1125–1133, 1991.

ROSETO, R.N.; POSADA, S.L.O. Modelación de la cinética de degradación de alimentos para rumiantes, **Revista Colombiana de Ciencias Pecuarias**, v.20, p.174-182, 2007.

RUIZ, R. L. **Microbiologia Zootécnica**, São Paulo: Roca, 1992, 314 p.

RUSSELL, J.B.; STROBEL, H.J. Effects of additives on in vitro ruminal fermentation: a comparison of monensin and bacitracin, another gram-positive antibiotic, **Journal of Animal Science**, v. 66, p. 552-558, 1988.

RUSSELL, J. B. A proposed mechanism of monensin action in inhibiting ruminal bacterial growth: Effects on ion flux and protonmotive force. **Journal of Animal Science**, v.64, p.1519–1525, 1987.

RUSSEL, J.B.; STROBEL, H.J. Effect of ionophores on ruminal fermentation, **Applied and Environmental Microbiology**, Washington, v.55, n.1, p.1- 6, 1989.

SAUER, F.D.; KRAMER, J.K.G.; CANTWELL, W.J. Antiketogenic effects of monensin in early lactation. **Journal of Dairy Science**, v.72, p.436–442, 1989.

SCHELLING, G.T. Monensin mode of action in the rumen, **Journal of Animal Science**, v. 58, p.1518-1527, 1984.

SCHINK, B.; STAMS, A.J.M. Syntrophism among prokaryotes, In: DWORKIN M, FALKOW S, ROSENBERG E, SCHLEIFER KH, STACKEBRANDT E (eds), **The Prokaryotes: an evolving electronic resource for the microbiological community**, 3rd ed, New York: Springer-Verlag, p.309-335, 2006.

SHARP, R.; ZIEMER, C.J.; STERN, M.D.; STAHL, D.A. Taxonspecific associations between protozoal and methanogen populations in the rumen and a model rumen system. **FEMS Microbiology Ecology**, v.26, p.71–78, 1998.

SILVA, M.M.C.; RODRIGUES, M.T.; RODRIGUES, C.A.F.; BRANCO, R.H.; LEÃO, M.I.; MAGALHÃES, A.C.M.; MATOS, R.S. Efeito da suplementação de lipídios sobre a digestibilidade e os parâmetros da fermentação ruminal em cabras leiteiras, **Revista Brasileira de Zootecnia**, v.36, p.246-256, 2007.

SIMPSON, M.E. Effects of certain antibiotics in vitro cellulose digestibility and volatile fatty acid production by ruminal microorganisms. **Journal of Animal Science**, v.47, Sup.1, p. 429 (Abstr.), 1980.

SISSONS, J.W.; THURSTONS, M.; SMITH, R.H. Reticular myoelectric activity and turnover of rumen digesta in the growing steer. **Canadian Journal of Animal Science**, v.64, p.70-71, 1984.

SMITH, W.A.; HARRIS, JR.B. The influence of type of forage on the production response of lactation dairy cows supplemented with different types of fat, **The Professional Animal Scientist**, v.8, p.7-21, 1993.

SOLIVA, C.R.; MEILE, L.; CIEŚLAK, A.; KREUZER, M.; MACHMÜLLER A. Rumen simulation technique study on the interactions of dietary lauric and myristic acid supplementation in suppressing ruminal methanogenesis. **British Journal of Nutrition**, v.92, p.689-700, 2004.

SOUZA, C.F. **Biodigestão anaeróbia de dejetos suínos: obtenção de dados e aplicação no desenvolvimento de um modelo dinâmico de simulação da produção de biogás**, 2001, Tese (doutorado em ciências) - Faculdade de Ciências agrárias e veterinárias, Universidade Estadual Paulista, Jaboticabal: UNESP, 2001.

SOWERS, K.R. Methanogenic Archaea: an overview, In: SOWERS, K.R.; SCHREIER, H.J, (Ed.), **Archaea, a Laboratory Manual Methanogens**, New York: Cold Spring Harbor Laboratory Press, p.3-13, 1995.

STEIL, L. **Avaliação do uso de inóculo na digestão anaeróbia de resíduos de aves de postura, frangos de corte e suínos**, 2001, 108 p, Dissertação (Mestrado) - Universidade Estadual Paulista, Araraquara, 2001.

STEWART, C.S.; BRYANT, M.P. The rumen bacteria, In: HOBSON P.N, (Ed.), **The Rumen Microbial Ecosystem**, New York: Elsevier Applied Science, p.21-75, 1988.

STEWART, C.S.; DUNCAN, S.H.; JOBLIN, K.N. Antibiotic manipulation of rumen microflora, The effects of avoparcin and monensin on the release of tritium from labeled cellulose by *Bacteroides succinogenes* in the rumen fungus *Neocallimastix frontalis*, In S, P. 1987.

TAMMINGA, S.; DOREAU, M. Lipids and rumen digestion, In: JOUANY, J.P. (Ed.) **Rumen microbial metabolism and ruminant digestion**, Paris: Institut National de la Recherche Agronomique, p.151-164, 1991.

THIVEND, P.; JOUANY, J.P. Effect of lasalocid sodium on rumen fermentation and digestion in sheep, **Reproduction Nutrition Development**, v.23, p.817-828, 1983.

TOWNE, G.; NAGARAJA, T.G.; BRANDT JR. R.T. et al, Ruminal ciliated protozoa in cattle fed finishing diets with or without supplemental fat, **Journal of Animal Science**, v.68, p.2150-2155, 1990.

TYMENSEN, L.D.; BEAUCHEMIN, K.A.; MCALLISTER, T.A. Structures of free-living and protozoa-associated methanogen communities in the bovine rumen differ according to comparative analysis of 16S rRNA and mcrA genes. **Microbiology**. v.158, p.1808-1817, 2012.

UEDA, K.; FERLAY, A.; CHABROT, J.; LOOR, J.J.; CHILLIARD, Y.; DOREAU, M. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage:concentrate ratios, **Journal of Dairy Science**, v.86, p. 3999-4007, 2003.

USHIDA, K.; KAYOULI, C.; DE SMET, S.; JOUANY, J.P. Effect of defaunation on protein and fibre digestion in sheep fed on ammoniatreated straw-based diets with or without maize, **British Journal of Nutrition**, v.64, p.765-775, 1990.

USHIDA, K.; NEWBOLD, C.J.; JOUANY J.P. Interspecies hydrogen transfer between the

rumen ciliate *Polyplastron multivesiculatum* and *Methanosarcina barkeri*. **The Journal of General and Applied Microbiology**, v.43, p.129–131, 1997.

VAN NEVEL, C. J.; DEMEYER, D.I. Effect of monensina on rumen metabolism in vitro, **Applied and Environmental Microbiology**, v.34, p.251-257, 1977.

VAN NEVEL, C.; DEMEYER, D.I. Manipulation of rumen fermentation, In: HOBSON, H.D. (Ed.) **The rumen microbial ecosystem**, New York: Elsevier Science, p.387-443, 1988.

VAN NEVEL, C.J.; DEMEYER, D.I. Control of rumen methanogenesis, **Environmental Monitoring and Assessment**, v.42, p.73-97, 1996.

VAN SOEST, P.J. **Nutritional ecology of the ruminant**, 2,ed, New York: Cornell University Press, 1994, 476p.

VÁRADYOVÁ, Z.; KIŠIDAYOVÁ, S.; SIROKA, P.; JALČ, D. Fatty acid profiles of rumen fluid from sheep fed diets supplemented with various oils and effect on the rumen ciliate population. **Czech Journal of Animal Science**, v. 52, p. 399-406, 2007.

VEIRA, D.M.; IVAN, M.; JUI, P.Y. Rumen ciliate protozoa: Effects on digestion in the stomach of sheep. **Journal of Dairy Science**, v.66, n.5, p.1015-1022, 1983.

VEIRA, D.M. The role of ciliate protozoa in nutrition of the ruminant, **Journal of Animal Science**, v.63, p.1547-1560, 1986.

VICINI, J.L.; BRULLA, W.J.; DAVIS, C.L.; BRYANT, M.P. Quin's oval and other microbiota in the rumen of molasses-fed sheep. **Applied and Environmental Microbiology**, v.47, p.219–221, 1987.

VOGELS, G.D.; HOPPE, W.F.; STUMM, C.K. Association of methanogenic bacteria with rumen ciliates, **Applied and Environmental Microbiology**, v.40, n.3, p.608-612, 1980.

WAGNER, J.R.; GREEN, H.B.; SYMANOWSKI, J.T. et al. Effect of monensin on feed intake, body weight, and body condition in dairy cows. **Journal of Dairy Science**, v.82 (Suppl. 1), p.75, 1999.

WAINMAN, F.W.; DEWEY, P.J.S. The energy value to sheep of saturated and unsaturated fat fed singly or in combination, **Animal Production**, v. 44, p.227, 1987.

WALLACE, R.J.; JOBLIN, K.N. Proteolytic activity of a rumen anaerobic fungus, **FEMS Microbiology Letters**, v. 29, p.19-25, 1985.

WALLACE, R.J.; CHENG, K.J.; CZERKAWSKI, J.W. Effect of monensin on fermentation characteristics of the artificial rumen. **Applied and Environmental Microbiology**, v. 40, p. 672-674, 1980.

WALLACE, R.J. Ruminant Microbiology, Biotechnology, and Ruminant Nutrition: Progress and Problems'. **Journal of Animal Science**, v. 72, p. 2292-3003, 1994.

WHITE, T.W.; GRAINGER, R.B.; BAKER, F.H.; STROUD, J.W. Effect of supplemental fat on digestion and the ruminal calcium requirement of sheep, **Journal of Animal Science**, v. 17, p.797-803, 1958.

WHITELAW, F.G.; EADIE, J.M.; MANN, S.O.; REID, R.S. Some effects of rumen ciliate protozoa in cattle given restricted amounts of a barley diet, **British Journal of Nutrition**, v.27, p.425-437, 1972.

WHITELAW, F.G.; EADIE, J.M.; BRUCE, L.A.; SHAND, W.J. Methane formation in faunated and ciliate-free cattle and its relationship with rumen volatile fatty acid proportions. **British Journal of Nutrition**, v.52, p.261-75, 1984.

WILLIAMS, A.G.; COLEMAN, G.S. The Rumen protozoa, In: HOBSON, H.D, (Ed.) **The rumen microbial ecosystem**, New York: Elsevier Science, 1988, p.77-128.

WILLIAMS, A.G.; COLEMAN, G.S. The Rumen Protozoa. New York: **Springer-Verlag**. 1992.

WOLIN, M.J.; MILLER, T.L.; STEWART, C.S. Microbe-microbe interactions, in **The Rumen Microbial Ecosystem**, eds Hobson P. N., Stewart C. S., editors. London: Blackie, p. 467-491. 1997.

WU, Z.; HUBER, J.T. Relationship between dietary fat supplementation and milk protein concentration in lactating cows: A review, **Livestock Production Science**, v.39, p.141-155, 1994.

YANG, S.L.; BU, D.P.; WANG, J.Q.; HU, Z.Y.; LI, D.; WEI, H.Y.; ZHOU, L.Y.; LOOR, J.J. Soybean oil and linseed oil supplementation affect profiles of ruminal microorganisms in dairy cows. **Animal**, v.3, p.1562-1569, 2009.

YANG, S.T.; GUO, M. Kinetics of methanogenesis from whey permeate in packed bed immobilized cells bioreactor. **Biotechnol Bioeng**, v.36, p.427-436, 1990.

YOKOYAMA, M.T.; JOHNSON, K.A. Microbiology of the rumen and intestine, In: CHURCH, D.C. (Ed.) **The ruminant animal, Digestive physiology and nutrition**, New York: John Wiley & Sons, 1993, p.125-144.

ZHANG, C.M.; GUO, Y.Q.; YUAN, Z.P.; WU, Y.M.; WANG, J.K.; LIU, J.X.; ZHU, W.Y. Effect of octadeca carbon fatty acids on microbial fermentation, methanogenesis and microbial flora in vitro, **Animal Feed Science and Technology**, v.146, p.259-269, 2008.

CHAPTER III.
DIET SUPPLEMENTATION WITH VEGETABLE OILS AND
MONENSIN: EFFECT ON RUMINAL FERMENTATION, METHANE
PRODUCTION, MICROBIAL PROTEIN SYNTHESIS AND
NUTRIENT DIGESTIBILITY

3. DIET SUPPLEMENTATION WITH VEGETABLE OILS AND MONENSIN: EFFECT ON RUMINAL FERMENTATION, METHANE PRODUCTION, MICROBIAL PROTEIN SYNTHESIS AND NUTRIENT DIGESTIBILITY

Abstract: The present study aimed to evaluate the effect of vegetable oils and monensin supplementation in diets on ruminal fermentation, methane production, microbial protein synthesis and total tract apparent digestibility of nutrients in Nellore cows. Eight Nellore cows (494 ± 105 kg of BLW) non pregnant and non-lactating, ruminally cannulated (10 cm of diameter and 7.5 cm of thickness) were randomly distributed to one of eight experimental diets: Control (CONT), diet without lipid and monensin inclusion, canola (CAN), soybean (SOY) and sunflower (SUN). The inclusion of lipid sources in the diet was 3.5% of DM as oil. Besides vegetable oils inclusion, each cow in a square daily received 300 mg (approximately 32 mg / kg DM) of sodium monensin (Rumensin® 200, Elanco Saúde Animal, Brazil) during the whole experimental period, delivered twice a day (150 mg at 08h00 and 150 mg at 16h00), mixed with the diet. The experimental design was a replicated 4 x 4 Latin square, in a factorial arrangement of treatments 2 x 4 (regarding inclusion or not of sodium monensin and lipids source inclusion in diets). Each experimental period had 26 days (20 days for diet adaptation and 6 days for data collection) totalizing 104 days. Neutral detergent fiber digestibility was decreased by 9.9% and 8.6% in SUN and SOY treatments, respectively, compared to control. Crude protein digestibility was increased when animals received the treatments CAN and SOY (7.8% and 6.1%, respectively). Monensin supplementation decreased mean ruminal pH by 4.8%, when compared to no monensin addition. Methane emission was reduced by SOY treatment in 22.2%. Methane emission was also reduced in 17.04% with monensin addition in diets ($P = 0.0041$). Treatments CAN and SOY reduced butyric acid (32.4 and 30.3%, respectively). Effect of interaction was observed for total SCFA, where in the absence of monensin addition in diet, CAN treatment reduced this variable by 31.52% when compared to the control treatment. Whereas, in the presence of monensin, soybean oil improved the response, leading to a reduction of 36.97% in total SCFA. Treatments SOY and SUN reduced total counts of protozoa and the genus *Entodinium*. While that, all the lipid sources reduced protozoa of the genus *Isotricha* and *Diplodininae*. Ruminal $\text{NH}_3\text{-N}$ concentrations at time 0 and 30 minutes of incubation were increased by 25.6% and 26.3%, respectively, by monensin addition. Lipid sources in the form of oil, as well as the antagonistic or synergistic effect of the association between monensin and vegetable oils result in changes in ruminal fermentation parameters, stimulating efficient metabolic

pathways to reduce methane emissions. The present study demonstrates that the combination of monensin with a source of omega-3 polyunsaturated fatty acid, such as soybean oil, results in a potentialize effect (synergism) on the reduction of total short chain fatty acids (g / kg / d) and the release of energy in the form of acetic acid expressed in percentage of digestible energy.

Key words: Ammoniacal nitrogen. Methane. Microbial protein. Rumen protozoa. Ruminal fermentation.

3.1 INTRODUCTION

The increasing concentration of greenhouse gases (GHG) in the atmosphere as a result of human activities, such as agricultural expansion (especially livestock and rice cultivation), industrial activities, exploration and use of fossil fuels, as well as, the production and management of residues (waste) represent the main cause of global warming and, consequently, climate change (LASSEY, 2008). The most important greenhouse gases resulting from such activities include carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O) and fluorinated gases (USEPA, 2007).

Methane is an important GHG with a global warming capacity 26 times greater than CO₂ (IPCC, 2007). It is produced under anaerobic conditions during the degradation of organic materials by certain microorganisms. Methane produced from enteric fermentation represents between 17 and 37% of anthropogenic GHG emissions (BEAUCHEMIN et al., 2010; SEJIAN et al., 2011), corresponding to approximately 7.1 Gt equivalente of CO₂ per year (GERBER et al., 2013). The production of enteric CH₄ by cattle represents a significant loss of energy in the diet. Generally, cattle lost between 2 and 15% of their energy intake as eructed CH₄, which correspond to 250-500 liters of CH₄ per animal per day (JOHNSON, 1995a; JOHNSON, 1995b).

Nutrition mitigation strategies can be classified into two major groups. The first strategy includes better utilization of dietary nutrients in order to decrease CH₄ emission and thus increase animal productivity. The second strategy consists of redirecting rumen fermentation to a lower total production of CH₄ (IQBAL et al., 2008), thus considering the metabolic pathways involved in the formation and use of hydrogen (H₂), with concomitant stimulation of pathways that consume H₂ to avoid the negative effects of increasing the partial pressure of H₂ in the rumen (McALLISTER; NEWBOLD, 2008; MARTIN et al.,

2009). Recent studies have evaluated nutritional compounds capable of reducing the production of CH₄ in the rumen (BEAUCHEMIN et al., 2008; PATRA, 2012). However, most of them have either inconsistent efficacy or are toxic to host animals at concentrations that are effective in mitigating CH₄ production (BEAUCHEMIN et al., 2008; MARTINE et al., 2010; PATRA, 2012). In the last years, vegetable oils have been widely evaluated in cattle diets, showing a great potential in reducing CH₄ emissions in dairy cows (MARTIN et al., 2010; HOLLMANN et al., 2012) or beef cattle (BEAUCHEMIN; MCGINN, 2006) due to their different mechanisms of action that contribute to the reduction of the methanogenesis process, such as: the substitution of fermentable organic matter by lipids (KNAPP et al., 2014), the reduction of methanogenic activity by the presence of medium chain fatty acids (MACHMULLER et al., 2003; MACHADO et al., 2011), mainly lauric and myristic acids (ODONGO et al., 2007; HOLLMANN et al., 2012), the toxic effect of dietary supplements rich in long chain fatty acids or vegetable oils rich in monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) (BEAUCHEMIN; MCGINN, 2006) on the cell membrane, particularly Gram-positive bacteria (NAGARAJA et al., 1997), the changes observed in the ruminal fermentation of acetate for propionate production and the direct inhibitory effects on methanogenic and protozoa microorganisms (SILVA et al., 2007; MARTIN et al., 2010).

In contrast, for additives such as monensin, *in vitro* and *in vivo* studies have shown that the anti-methanogenic effects of ionophores are more related to the inhibition of methane precursors formation (formate and H₂) than a direct effect on methanogenic population, since these are more resistant to ionophores than the bacteria that produce and supply H⁺ (NAGARAJA et al., 1997; RUSSELL; HOULIHAN, 2003). However, despite the effective ability of ionophores to decrease CH₄ and to change the fermentation of acetate to propionate, it has a short-term efficacy. Johnson & Johnson (1995) and Guan (2006) showed that the effect of methane production suppression lasted less than 30 days. On the other hand, several studies have also documented the resistance of Gram-positive bacteria to monensin (NEWBOLD et al., 1993; RAVVA et al., 2013; KIM et al., 2014).

By what was previously exposed, changes in ruminal microbial activity and fermentation variables associated to decreased ruminal CH₄ production and increased efficiency of microbial protein synthesis, when vegetable oils were included in combination with monensin in cattle diets, are not yet well characterized. Therefore, it is of great importance to identify if the combination of these factors generates a synergistic or additive type effect in the modulation of the ruminal fermentation or if on the contrary, the

interaction of these results in an antagonistic type effect. It is expected that lipid sources, which can redirect the energy of the rumen to the posterior digestive tract and have a direct effect on methanogenic rumen population, as well as a small effect as a H₂ drain, associated to monensin, which can direct the H₂ generated from ruminal metabolism, may modify ruminal metabolism and fermentation variables, contribute with a substantial decrease in methanogenesis and with the consequent reduction of enteric CH₄ production. Further, sources of unsaturated fatty acids, when combined with monensin, are expected to exhibit synergistic effects (potentialized), or at least additive effects, more substantial in enhancing the efficiency of microbial protein synthesis and in reducing greenhouse gas emission by ruminants.

The objective of the present experiment was to evaluate the effect of supplementation with vegetable oils and monensin in cattle diets on ruminal fermentation, characterization of rumen protozoa population, methane production, microbial protein synthesis and total tract apparent nutrient digestibility.

3.2 MATERIALS AND METHODS

The present study was conducted in the Laboratory of Ruminant Nutrition of the College of Veterinary Medicine and Animal Science, University of Sao Paulo (USP), Pirassununga, Brazil. The experiment was conducted according the guidelines established by the Ethics Committee in the Use of Animals of the College of Veterinary Medicine and Animal Science, under the protocol N° 9236160216, in respect of ethical principles of animal experimentation for care and use of animals.

3.2.1 Animals, Treatments and Experimental design

Eight Nellore cows (494 ± 105 kg of body live weight) non pregnant and non-lactating, ruminally cannulated (10 cm of diameter and 7.5 cm of thickness) were randomly distributed to one of eight experimental diets, isoenergetic (1.2 Mcal of NE_g/kg of DM) and isoproteic (14% of CP), that differ by the lipid source used and the addition or not of sodium monensin (Rumensin® 200, Elanco Animal Health, Brasil). The treatments were: Control (CONT), diet without lipid and monensin inclusion, canola (CAN), soybean (SOY) and sunflower (SUN). The inclusion of lipid sources in the diet was 3.5% of DM as oil. Besides vegetable oils inclusion, each cow in a square daily received 300 mg

(approximately 32 mg / kg DM) of sodium monensin (Rumensin® 200, Elanco Saúde Animal, Brazil) during the whole experimental period, delivered twice a day (150 mg at 08h00 and 150 mg at 16h00), mixed with the diet.

The experimental design was a replicated 4x4 Latin square, in a factorial arrangement of treatments 2x4 (regarding inclusion or not of sodium monensin and lipids source inclusion in diets). The experimental unit was the animal inside each period, totalizing 32 experimental units. Each experimental period had 26 days (20 days for diet adaptation and 6 days for data collection) totalizing 104 days.

Diets were offered twice a day, at 08h00 and 16h00 as a total mixed ration (60:40, forage:concentrate ratio), for *ad libitum* intake (5 - 10% of leftovers). In all diets the forage source used was corn silage. Forage and concentrate feeds were weekly sampled for DM content determination and diets were adjusted considering their variation in DM content. The amount of forage and concentrate daily offered was adjusted depending on leftovers from the day before. Diets were formulated to attend nutritional requirements for beef cattle, according NRC (2000). The proportion of ingredients and chemical composition are described at Table 4.

For determination of fatty acids profile of oils included in experimental diets (Table 5), the extraction was performed by methodology described by FOLCH et al. (1957), by sample homogenization with a solution composed by chloroform and metanol 2:1 in homogenizer Ultra Turrax Marconi®. Lipids were isolated after the addition of NaCl 1.5% solution. For methylation, methyl esters were formed according methodology described by Kramer et al. (1997). Fatty acids were quantified by gas chromatography (CG-2010 Plus - Shimadzu, auto injetor AOC 20i), using cappillary column SP-2560 (100 m × 0.25 mm of diameter with 0.02 mm of thickness, Supelco, Bellefonte, PA). The initial temperature of column was 45°C, with progressive heating until reaching 175°C, remaining for 27 minutes. After, a further increase of 4 °C/min was started up to 215°C, remaining for 35 minutes. Hydrogen (H₂) was used as a carrier gas with flux of 40 cm³/s. The temperature used by the flame ionization detector (FID) was 250°C, H₂ flow of 40 mL/min, air flow of 400 mL/min, make-up of 30 mL/min kPa (N₂) and sampling rate of 40 msec. The total time of each run per sample (stop time) was 86 minutes.

Fatty acids were identified according retention time of methyl esters of samples using the standard solutions 463 Nu-Chek®, vaccenic acid C18:1 trans-11 (V038-1G, Sigma®), C18:2 trans-10 cis-12 (UC-61M 100mg), CLA and C18:2 cis-9, trans-11 (UC-60M 100mg) and tricosanoic acid (Sigma®). Fatty acids were quantified by normalization

of the peak area of the methyl esters, using the GS solution software 2.42®. Fatty acids were expressed as percentage of total methyl ester quantified.

Table 4 - Proportion of ingredients and chemical composition of experimental diets expressed as percentage of dry matter (% DM).

Item	Experimental diets ¹			
	CONT	CAN	SOY	SUN
Ingredients, % DM				
Corn silage	60.9	61.9	61.9	61.9
Ground corn grain	22.6	14.0	14.0	14.0
Soybean meal	13.4	17.5	17.5	17.5
Vegetal oil	-	3.5	3.5	3.5
White salt	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.1	0.1	0.1	0.1
Limestone	0.5	0.5	0.5	0.5
Mineral mixture ²	2.0	2.0	2.0	2.0
Chemical composition				
Dry matter, %	54.1	53.8	53.8	53.8
CP, % of DM	14.6	14.9	14.6	14.1
RDP, % of CP ³	66.8	69.4	69.4	69.4
RUP, % of CP ³	33.2	30.6	30.6	30.6
NDF, % of DM	40.6	38.1	41.8	40.3
ADF, % of DM	26.0	24.7	29.4	29.6
NFE, % of DM	34.6	33.3	31.7	30.0
Ash, % of DM	7.1	7.3	7.3	7.3
Ca, % of DM	0.89	1.07	1.07	1.07
P, % of DM	0.30	0.29	0.29	0.29
EE, % of DM	3.1	6.4	6.3	6.6
TDN, % of DM	76.3	78.1	78.0	77.6
NE _m , Mcal/kg of DM ⁴	1.83	1.88	1.88	1.87
NE _g , Mcal/kg of DM ⁴	1.20	1.25	1.24	1.23

¹CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil. Monensin was included in respective diets at 300 mg per animal per day – approximately 32 mg/kg of DM; ²Mineral and vitamin premix, composition per kg of product: 200 g of Ca, 60 g of P, 20 g of S, 20 g of Mg, 70 g of Na, 15 mg of Co, 700 mg of Cu, 700 mg of Fe, 40 mg of I, 1,600 mg of Mn, 19 mg of Se, 2,500 mg of Zn; ³Values estimated by the software *Nutrient Requirements of Beef Cattle: Seventh Revised Edition (2000)*. ⁴ME (Metabolizable energy) = [(TDN kg x 0.04409) x 0.82] x 100 (NRC, 2000); equations to convert ME values into NE_m and NE_g (Mcal/kg DM) have been reported by Garrett (1980) and NRC (2000).

Table 5. Fatty acids profile of oils used in experimental diets.

Fatty acid ¹	Canola oil	Sunflower oil	Soybean oil
Lauric, C12:0	0.021	0.008	0.001
Myristic, C14:0	0.061	0.065	0.079
Palmitic, C16:0	4.445	6.148	11.09
Palmitoleic, C16:1 trans 9	0.036	0.013	0.008
Palmitoleic, C16:1 cis 9	0.219	0.091	0.080
Heptadecanoic, C17:0	0.080	0.038	0.071
Heptadecenoic, C17:1 cis 10	0.115	0.027	0.042
Stearic, C18:0	2.334	3.870	3.330
Oleic, C18:1 n-9 cis	56.23	37.67	21.45
Vaccenic, C18:1 cis 11	6.294	0.132	2.389
Linoleic, C18:2 n-6 cis	18.69	50.00	53.38
γ -linolenic, C18:3 n-6	0.421	0.010	0.097
Linolenic, C18:3 n-3	8.546	0.242	6.427
Araquidic, C20:0	0.020	-	0.317
Eicosenoic, C20:1 cis 5	0.062	0.007	0.022
Eicosenoic, C20:1 cis 8	0.365	0.004	0.083
Eicosadienoic, C20:2 n-6 cis 11,14	0.045	0.005	0.032
Eicosatrienoic, C20:3 n-6 cis 8, 11, 14	0.363	0.662	0.421
Eicosatrienoic, C20:3 n-3 cis 11, 14, 17	0.043	0.014	0.005
Heneicosanoic, C21:0	0.011	-	0.020
Tricosanoic, C23:0	0.021	0.019	0.038
Eicosapentaenoic, C20:5 n-3 cis (EPA)	0.162	0.188	0.125
Docosatrienoic, C22:3 n-3 cis 13, 16, 19	0.123	0.015	0.015
Docosatetraenoic, C22:4 n-6	0.009	-	-
Docosahexaenoic, C22:6 n-3 cis (DHA)	0.008	-	0.005
PUFAS ²	28.41	51.14	60.51
MUFAS ³	63.32	37.94	24.09
SFAS ⁴	7.011	10.16	14.95
n-3	8.882	0.459	6.577
n-6	19.52	50.67	53.93
n-6/n-3	2.197	110.3	8.199

¹Fatty acids expressed in percentage (%) of the sample; ²PUFA: Polyunsaturated fatty acids; ³MUFA: Monounsaturated fatty acids; ⁴SFA: Saturated fatty acids.

3.2.2 Dry matter intake, total tract apparent digestibility of dry matter and its fractions and nutrients excretion

Dry matter intake (DMI) was daily measured during the last six days of each experimental period. Leftovers were collected and weighed every morning at 06h30, in order to adjust the quantity of forage and concentrate daily offered. When there was no

leftover, offered diet was raised in 10%. If there was a surplus between 5 - 10%, diet was kept at the same level and if the leftover was higher than 10%, offered diet was reduced in 10%. Dry matter intake was estimated by the difference between the quantity of diet supplied in one day and leftover, collected and weighed in the morning following the offering, multiplied by the percentage of dry matter of the diet.

Total tract DM apparent digestibility and its fractions (CP, EE, NFE, NDF, ADF and GE) was determined by *in vivo* methodology with titanium dioxide (TiO₂), according to Titgemeyer et al. (2001). For this analysis, between day 15 and day 24 of each experimental period, TiO₂ (15 g/animal/d) was administered via ruminal cannula, divided into two 7.5 g/animal/d. The first five days were used for external marker adaptation and the last five days for feces collection (200 g of wet weight approximately), performed manually, directly from the rectum, twice a day (at 08h00 and 16h00). Composite samples were stored in freezer (-20°C) until the moment of determination of TiO₂ concentration, according Myers et al. (2004). Coefficients of DM apparent digestibility (CAD_{DM}) and its nutrients (CAD_N) were estimated on basis on TiO₂ concentration of diet and feces, as well as, the nutrients content present on diet and feces collected, using the following equations:

$$CAD_{(DM)} = 100 - \left[100 \left(\frac{\% TiO_2^d}{\% TiO_2^f} \right) \right] \quad (1)$$

$$CAD_{(N)} = 100 - \left[100 \left(\frac{\% TiO_2^d}{\% TiO_2^f} \right) \times \left(\frac{\% Nf}{\% Nd} \right) \right] \quad (2)$$

where:

CAD_(DM) = Coefficient of apparent digestibility of DM;

CAD_(N) = Coefficient of apparent digestibility of nutrients;

% TiO₂d = Percentage of titanium dioxide in diet;

% TiO₂f = Percentage of titanium dioxide in feces;

% Nd = Percentage of nutrient in diet;

% Nf = Percentage of nutrient in feces.

Dry matter and nutrients excretion (kg/d) were determined from DM and is fractions coefficient digestibility, as well as, dry matter intake of each nutrient, as indicated in the following equation:

$$\text{Excretion}_{(n)} = \frac{(100 - \text{CAD}_n) \times \text{Intake of DM or nutrient}}{100} \quad (3)$$

where:

Excretion_(n) = Excretion of DM or nutrient;

CAD_(n) = Coefficient of apparent digestibility of DM or nutrient;

Samples of feed used in diets and of collected feces (approximately 200 g, per treatment and per period), were dried at 55°C for 72 h in a forced air circulation oven and ground (1.0 mm particle size in Willye type mill) for determination of dry matter (DM-Method 930.15), mineral matter (MM-Method 942.05), crude protein (CP-Method 984.13) content by total N determination (N x 6.25) using the micro-Kjeldahl technique, calcium (Ca-Method 968.08) and phosphorus (P-Method 965.17), according AOAC (2000), ether extract (EE-Method Am 5-04) by ANKOM XT15 Extractor® equipment, according AOCS (2005), neutral detergent fiber (NDF) and acid detergent fiber (ADF), according Van Soest et al. (1991). These samples were performed with the objective to evaluate nutrient composition of offered diet, as well as, intake and digestibility of each nutrient in diet.

3.2.3 Variables and products of ruminal fermentation

3.2.3.1 Ruminal pH

Ruminal pH was continuously measured during 24 h (every 10 min) on day 25 of each experimental period using a pH data logger (Model T7-1 LRCpH, Dascor®, Escondido, CA, USA) (PENNER et al., 2006). Attached on the data logger, 2 weighs of 900 grams each were used to maintain the device in the position in the rumen ventral sacral. Each electrode was standardized using pH 4.0 and 7.0 standards at the beginning and end of each session. The pH data were recorded as mean, maximum, and minimum pH, this included the area under the curve and duration of time in which pH was below 5.8; 6.0 and 6.2. The pH 5.8 indicates the threshold of cases of subacute ruminal acidosis (PENNER et al., 2007), and the pH 6.0 to 6.2 are thresholds indicative of healthy ruminal

conditions (PENNER; BEAUCHEMIN, 2010). The area under the curve was calculated by multiplying the absolute value of deviations in pH by the time (min) spent below the established threshold for each measure, and it was then divided by 60 and expressed as pH unit $\times$ hour (MOYA et al. 2011).

3.2.3.2 Ex situ ruminal fermentation technique (micro-rumen)

The production of short chain fatty acids (SCFA), methane (CH_4) and ammonia nitrogen ($\text{NH}_3\text{-N}$) were determined by the ex situ ruminal fermentation technique (RODRIGUES et al., 2012; PERNA JUNIOR et al., 2017). The principle of the ex situ technique consists in placing ruminal contents samples in penicillin type vials (micro-rumen) incubated in a water bath (39°C), simulating the rumen conditions of the animals (presence of microorganisms, anaerobiosis, temperature of 39°C , natural saliva of the animal, physiological rumen pH), for 30 minutes. Finally, after the fermentation blockage, the measurements of the final products of the ruminal fermentation (CH_4 and SCFA) are performed and the energy losses of CH_4 are expressed in relation to the other fermentation products.

Ruminal contents samples were collected on day 25 of each experimental period through rumen cannula before (0) and, 3, 6, 9 and 12 hours after morning feeding. On this day, animals were only fed once in the morning (08h00) and the second meal was only offered after the last rumen samples collection. Approximately 300 mL of rumen fluid (liquid phase – obtained by a probe coupled to a vacuum pump) and 300 g of rumen solid content (solid phase - withdrawn manually) were collected at each sampling time from three different regions of the rumen (dorsal, ventral and medial region). Samples were packed in a plastic container. The two fractions were mixed and homogenized in the ratio of 66% liquid phase and 33% solid phase.

For each animal and at each sampling time, four penicillin-type bottles (50 mL clear glass vial, Frascolex, São Paulo, Brazil) were prepared. Two flasks (representing 2 replicates - T_{30}) were used for incubation for 30 minutes in a thermostatic bath (39°C) and two of them (which also represented 2 repetitions - T_0) were used as blanks (immediate inactivation under pressure and temperature - 0 minutes of incubation). Thirty mL of previously homogenized ruminal contents were poured through a funnel into each 50 mL glass vial. The flasks were then sealed with rubber stoppers and sealed with aluminum seals, using a crimping plier, later identified with plastic seals. The flasks were then

flushed with CO₂ by needles for gas input and output to ensure an anaerobic environment. After 30 minutes of incubation of the T₃₀ flasks and immediately after the filling of the flasks considered as blanks (T₀), the fermentation process was inactivated by autoclaving (pressure and temperature for 15 minutes). After the vials had cooled, they were taken to measure the volume of gas produced and the concentrations of CH₄ and SCFA by gas chromatography. The measurement of the gas volume produced by the sample from the ruminal fermentation in the incubated (T₃₀) and non-incubated (blank, T₀) flasks was performed using a pressure transducer (Datalogger universal AG5000, Genesis SM®, Barueri, SP, Brazil) connected to a digital reader and a three-way valve. For measurement, a needle was connected to the three-way valve through the rubber cap. Immediately afterwards, the volume was measured by drawing the accumulated gases into the top of the bottle using the syringe connected to the third valve until a zero-pressure reading was obtained. The total volume of gas produced (mL) per sample was obtained by the sum of the volume recorded in the syringe coupled to the pressure transducer (mL) plus the head space of the flask. After measurement by the transducer, the determination of the CH₄ concentration in the incubated and non-incubated flasks was performed by gas chromatography (Trace 1300, Thermo Fisher Scientific®, Rodano, Milan, Italy), injecting 0.5 mL of gas from each sample in an environment with controlled temperature (25 °C), according to Kaminski et al. (2003).

The volume of liquid in the incubated (T₃₀) and non-incubated (T₀) flasks was calculated by the difference between the weight of the flask containing the sample after oven drying at 105°C for 24 h and the weight of the flask containing the sample before drying. The solid content of the vials (T₃₀ and T₀) was obtained by the difference in the weight between the vial containing the sample after drying and the weight of the empty vial (before filling with ruminal content sample). For determination of SCFA concentration (acetate, propionate and butyrate), a fraction of ruminal content of each vial was centrifuged at $2,000 \times g$ for 20 min. Then, 2.0 mL of the supernatant was added in test tubes containing 0.4 mL of formic acid and immediately frozen at -20°C until the time of analysis. The SCFA concentration was measured as suggested by Erwin et al. (1961) by gas chromatography (Focus GC, Thermo Scientific®, Rodano, Milan, Italy) using a glass column 1.22 m long and 0.63 cm in diameter packed with 80/120 Carbopack B-DA/4 % (Supelco, Sigma-Aldrich®, St. Louis, MO, USA).

The quantification of the CH₄ production was calculated by the multiplication of the total volume of the gas produced (mL) by CH₄ concentration in the gaseous phase

(mmol/mL) obtained in the incubated flask (T_{30}). The result was subtracted from the value produced in the non-incubated flask (T_0). The individual quantification of SCFA was determined by the multiplication of the net volume (mL) by the concentration of each SCFA (mmol/mL) obtained in the incubated flask and this value subtracted from that obtained in the flask without incubation (T_0). The production of CH_4 (4) and SCFA (5) were calculated by the following equations (RODRIGUES et al., 2012; PERNA JUNIOR et al., 2017):

$$\text{Prod. } CH_4 = (\text{Conc. } CH_4 \times \text{Total Vol. Gas})T_{30} - (\text{Conc. } CH_4 \times \text{Total Vol. Gas})T_0 \quad (4)$$

where:

Prod. CH_4 = Production of CH_4 at the moment between the injection of ruminal content in the flasks and the inactivation of the ruminal fermentation process;

Conc. CH_4 = Concentration of CH_4 (mmol/mL);

Total Vol. Gas = Total volume of gases obtained by the sum of the volume determined by the transducer pressure (mL) and the head space of the flask (mL);

T_{30} = Incubation time of 30 min (incubated flask);

T_0 = Incubation time of 0 min (blank flask).

$$\text{Prod. SCFA} = (\text{Conc. SCFA} \times \text{Total Vol. Liq.})T_{30} - (\text{Conc. SCFA} \times \text{Total Vol. Liq.})T_0 \quad (5)$$

where:

Prod. SCFA = Production of SCFA in the moment between the injection of ruminal content in the flask and the inactivation of the ruminal fermentation process;

Conc. SCFA = Concentration of SCFA (mmol/mL);

Total Vol. Liq. = Total volume of liquid in the flask obtained by the difference of weight before and after drying at forced air circulation (mL);

T_{30} = Incubation time of 30 min;

T_0 = Incubation time of 0 min (blank flask).

Thereafter, CH_4 and SCFA productions were expressed based on the solid content incubated in the flasks (grams or kilograms). After quantification of the fermentation products (CH_4 and SCFA) of the sample contained in each flask, each product was multiplied by its heat of combustion to express the production of CH_4 in relation to the

energy from the fermentation generated. Thus, the relative energy loss (REL) was the ratio of the energy in CH₄ produced to the sum of the energy contained in the quantified fermentation products (CH₄ and SCFA), expressed as a percentage. For this, the chemical values of the heat of combustion to be used were 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, for acetic (C₂), propionic (C₃), butyric (C₄) acids, CH₄ and CO₂, respectively. REL was calculated using the equation suggested by Rodrigues et al. (2012):

$$\text{PER (\%)} = \frac{\varepsilon\text{CH}_4}{\varepsilon\text{CH}_4 + \varepsilon\text{C}_2 + \varepsilon\text{C}_3 + \varepsilon\text{C}_4} \quad (6)$$

where:

REL = Relative energy loss (%);

εCH_4 = Methane energy (kcal/g or kcal/mol);

εC_2 = Acetic acid energy (kcal/g or kcal/mol);

εC_3 = Propionic acid energy (kcal/g or kcal/mol);

εC_4 = Butyric acid energy (kcal/g or kcal/mol).

3.2.3.3 Ammonia nitrogen concentration

For determination of ammoniacal nitrogen (NH₃-N) concentration, 2.0 mL of centrifuged samples from each vial were placed in test tubes containing 1.0 mL of sulfuric acid solution (H₂SO₄) 1 N and immediately frozen at -20 °C until the colorimetric analysis, according to the method described by Kulasek (1972) and adapted by Foldager (1977). The balance (mg/dL/h) was obtained by subtracting the concentration of NH₃-N (mg/dL) after 30 min of incubation (T30) by the concentration obtained at time 0 (T0, blank). With this procedure, it was possible to evaluate if the balance of ammonia production in the rumen was positive or negative. The balance was estimated by hour according the following equation:

$$\text{Balance of NH}_3\text{-N (mg/dL/h)} = [\text{Conc. 30 min (mg/dL)} - \text{Conc. 0 min (mg/dL)}] \times 2 \quad (7)$$

where:

Conc. 30 min (mg/dL) = Concentration of NH₃-N in incubated flasks for 30 min;

Conc. 0 min (mg/dL) = Concentration of NH₃-N in incubated flasks for 0 min (blanks).

3.2.4 Rumen protozoa counts

For total and differential rumen ciliate protozoa counts, 10 mL of ruminal contents were manually collected from three different regions of the rumen (dorsal, ventral and medial) and stored in glass vials containing 20 mL of formaldehyde at 18.5%. Samplings were performed on day 25 of each experimental period at before (0) and, 3, 6, 9 and 12 hours after morning feeding. Subsequently, 1 mL of the sample diluted with formaldehyde was stained with two drops of bright green 2% and allowed to rest for 4 hours. Then, 9 mL of 30% glycerol was added, homogenizing the mixture and the sample was diluted 30 times. With a pipette, the counting chamber was filled with the diluted sample, which, coupled under the microscope, 100 optical fields were counted through the reticulum, increasing by 100x. Differential count of protozoa was determined using a Neubauer Enhanced Bright-Line counting chamber (Hausser Scientific Partnership®, Horsham, PA, USA) coupled to the eyepiece of a microscope (Olympus model CH2®), according to Dehority (1993). Protozoa species identification were established ($\times 10^3/\text{mL}$), as well as, their proportions (%) in the sample. The ciliate ruminal protozoa were divided into two subclasses: *Holotricha* (genus *Isotricha*, *Dasytricha* and subfamily *Diplodiniinae*) and *Entodiniomorpha* (*Entodinium*).

3.2.5 Energy partition – Energy balance

In order to determine the release of energy throughout the digestive process, gross energy intake per animal (GEI, Mcal/kg/d) was initially calculated by multiplying DMI (kg/d) by the gross energy of the diet (Mcal/kg DM). For calculation of the energy release of C_2 , C_3 , C_4 and CH_4 (Mcal/animal/d) in the rumen, the production of these metabolites (g/kg/d) was multiplied by their combustion heat (Mcal/g) and then multiplied by the ruminal solid mass in kg (the ruminal solid mass was obtained from the disappearance rate analysis, in the next chapter).

Rumen energy release when expressed as % RER was obtained by dividing C_2 , C_3 , C_4 and CH_4 production (Mcal/animal/d) by GEI (Mcal/animal/d) and then multiplying the value obtained per 100. The release of energy in the rumen, expressed as percentage of digestible energy (DE) was obtained by the following formula:

$$\text{SCFA or CH}_4 \text{ (\%DE)} = 100 \times \frac{\text{SCFA ou CH}_4 \text{ (Mcal/animal/d)}}{\text{GEI (Mcal/animal/d)} - \text{GE feces (Mcal/d)}} \quad (8)$$

where:

SCFA or CH₄ (%DE) = Energy release in the rumen when expressed by percentage of digestible gross energy;

SCFA or CH₄ (Mcal/animal/d) = Production per animal of acetate, propionate and butyrate (Mcal/animal/d);

GEI (Mcal/animal/d) = Gross energy intake per animal in Mcal per day;

GE feces (Mcal/d) = Quantity of gross energy present in animal feces.

The release of CH₄ into the cecum and colon was considered as 5% of the total release of CH₄, as indicated by Dini et al. (2012), where they suggest that enteric CH₄ is mainly produced in the rumen (95%) and (5%) in the large intestine. Fermentation heat losses and microbial ATP were estimated from the SCFA produced according to Owens & Basalan (2016).

The release of energy in the intestine (Mcal/animal/d) was calculated from gross energy intake (Mcal/animal/d) and subtracting from C₂, C₃, C₄, CH₄, Heat loss by fermentation (HLF) +ATP release in the rumen (Mcal/animal/d) plus feces gross energy (Mcal/animal/d) and CH₄ release in the cecum and colon (Mcal/animal/d), following this equation:

$$\text{ERI (Mcal/animal/d)} = \text{GEI} - (\varepsilon\text{C}_2 + \varepsilon\text{C}_3 + \varepsilon\text{C}_4 + \text{GE feces} + \text{CH}_4, \text{CC} + \text{HLF} + \text{ATP}) \quad (9)$$

where:

ERI = Energy release in intestine (Mcal/animal/d);

GEI = Gross energy intake (Mcal/animal/d);

εC_2 = Release of acetic acid in the rumen (Mcal/animal/d);

εC_3 = Release of propionic acid in the rumen (Mcal/animal/d);

εC_4 = Release of butyric acid in the rumen (Mcal/animal/d);

GE feces = Gross energy in feces (Mcal/animal/d);

CH₄, CC = Release of methane in cecum and colon (Mcal/animal/d);

HLF = Heat loss by fermentation;

ATP = Microbial ATP.

The release of energy in intestine, expressed as %GE or %DE was obtained by dividing the energy released in the intestine (Mcal/animal/d) by GEI (Mcal/animal/d) or DE (Mcal/animal/d) and then multiplying the value obtained by 100. The energy released in feces, expressed as %GE was obtained by dividing the fecal energy content (Mcal/animal/d) by GEI (Mcal/animal/d) and then multiplying by 100.

3.2.6 Nitrogen compounds, microbial protein synthesis and nitrogen balance

Urine samples were collected from all cows on day 26 of each experimental period, every 6 hours (at 06h00, 12h00, 18h00 and 24h00) by vulva massage. Each collected urine sample was filtered and a 10 mL aliquot (spot sample) was immediately diluted in 40 mL of sulfuric acid 0.036 N, in order to reduce the pH to values below 3.0 to avoid nitrogen losses (VASCONCELOS et al. 2010), bacterial destruction of purine derivatives and precipitation of uric acid. All spot samples were stored at -20 °C in a single vial per animal and per experimental period, composing a single 24-hour sample. Subsequently, creatinine, urea, uric acid and allantoin were analyzed.

Creatinine concentration was evaluated by commercial kits (Bioclin® Ref K067), through the colorimetric enzymatic reaction through reaction with alkaline picrate in buffered medium. Uric acid was determined by enzymatic colorimetric reaction through uricase and peroxidase by using a commercial kit (Bioclin® Ref k139). Urea concentration was also determined by colorimetric enzymatic reaction, using a commercial kit (Bioclin® Ref k047). Conversion of values of urea to urea nitrogen was performed by multiplying the urea concentration by the factor 0.466, corresponding to the N content in the urea.

Daily urinary excretion of creatinine (EC) was estimated in relation to body live weight (BLW, kg) from the equation proposed by Chizzotti et al. (2004):

$$EC \text{ (mg/kg of BLW/d)} = 32.27 - 0.01093 \times BLW \quad (10)$$

Daily urine volume (L/cow/d) was obtained from daily urinary creatinine (mg/kg BLW/d), multiplying EC value by body live weight (kg) of each animal and dividing the result by the urine creatinine concentration value (mg/L) of the spot samples.

Allantoin in urine (ALAU) was determined according to the colorimetric method described by Chen & Gomes (1992), while the concentration of purine derivatives (PD, mmol/d) in the urine was obtained by the sum of uric acid and allantoin concentrations

(mmol/d). From these values, the percentage of allantoin of the purine derivatives (% ALA of PD), as the ratio between ALA concentration and PD, was calculated by multiplying the obtained value by 100. The absorbed microbial purines (Pabs, mmol/d) were calculated from the excretion of purine derivatives in the urine (DP, mmol/d), according to the equation adapted from Verbic et al. (1990):

$$\text{Pabs (mmol/d)} = \frac{[\text{PD} - (0.385 \times \text{BW}^{0.75})]}{0.85} \quad (11)$$

where:

Pabs = Microbial purines absorbed;

$0.385 \times \text{BW}^{0.75}$ = Endogenous excretion of purine derivatives in urine by kg of metabolic body weight per day.

0.85 = Recovery of absorbed purines as purine derivatives in urine;

Intestinal flow of microbial nitrogen compounds (Nmic, g of N/d) was calculated in relation to absorbed microbial purines (Pabs, mmol/d) as a microbial indicator, whose quantification was performed according to the equation described by Chen & Gomes (1992):

$$\text{Nmic (g/d)} = (70 \times \text{Pabs}) / (0.83 \times 0.116 \times 1000) \quad (12)$$

where:

70 = Content of N in purines (mg N/mmol);

Pabs = Microbial absorbed purines;

0.83 = Microbial purines digestibility;

0.116 = Ratio Purine N:Total N of rumen microorganisms.

From the values of Nmic (g/d) obtained, the value of nitrogen compounds in relation to body live weight of the animals (Nmic, mg/kg of BLW) was calculated. The efficiency of microbial protein synthesis (EPSmic) in g per day of Pmic/kg of Rumens degradable protein (RDP) was calculated by the formula:

$$\text{EPSmic} = \text{Pmic (g/d)} / \text{RDP (kg/d)} \quad (13)$$

where:

EP_{Smic} = Efficiency of microbial protein synthesis;

P_{mic} = Microbial protein obtained from the multiplication of the produced N-microbial (g/d) by the factor 6.25;

RDP = Rumen degradable protein.

Rumen degradable protein was calculated by:

$$\text{RDP} = [\text{CPI (kg/d)} \times \text{Real CP Deg (\%)}] / 100 \quad (14)$$

where:

CPI (kg/d) = Crude protein intake;

Real CP Deg (%) = Real crude protein degradability obtained from the nylon bag technique proposed by Ørskov et al. (1980), as indicated in chapter IV.

The EP_{Smic} was also calculated in relation to g of N/kg of fermentable organic matter (EP_{Smic}, g N/kg MO_{fer}), by the ratio of produced N-microbial (g/d) and the kg of fermentable OM, where this last variable was obtained by the following formula:

$$\text{MO}_{\text{fer}} = [\text{OMI (kg/d)} \times \text{Real DM Deg (\%)}] / 100 \quad (15)$$

where:

OMI (kg/d) = Organic matter intake;

Real DM Deg (%) = Real dry matter degradability obtained from rumen degradability analysis as indicated in chapter IV.

Nitrogen balance (NB) was calculated considering the amounts of nitrogen ingested (g/d, N-ingested) and excreted in feces (N-feces) and in urine (N-urine). Likewise, from these values, it was possible to calculate the retention of nitrogen compounds, which included the percentage of N consumed and absorbed, as well as N-retained (g/d) in the animal's organism. Accordingly, the N value retained was calculated as:

$$\text{N-retained (g/d)} = [\text{N-ingested (g)} - (\text{N-feces (g)} + \text{N-urine (g)})] \quad (16)$$

3.2.7 Statistical analysis

Results were analyzed by the Statistical Analysis System (Version 9.4, SAS Inst., Inc., Cary, NC, USA 2015), after verifying residue normality by Shapiro-Wilk test (PROC UNIVARIATE) and variance homogeneity by Hartley test. Dry matter intake, ruminal pH, energy partition, microbial protein synthesis and nitrogen balance data were submitted to variance analysis, which separated as sources of variation the effects of oil, square (monensin), period, as well as, animal within square. The variables were analyzed using the following mathematical model:

$$Y_{ijkl} = \mu + O_i + M_j + (O_i \times M_j)_{ij} + P_k + A_l(M_j) + e_{ijkl}$$

where:

Y_{ijkl} = Observation related to Oil (i) + Monensin (j) + Oil (i) x Monensin (j) + Period (k) + Animal (l) inside Monensin (j) + random error associated to each observation (e_{ijkl});

μ = Average mean associated to the variable;

O_i = Fixed effect of oil i;

M_j = Fixed effect of monensin j;

$(O_i \times M_j)_{ij}$ = Interaction effect between oil (i) and monensin (j);

P_k = Random effect of period k;

$A_l(M_j)$ = Random effect of animal (l) inside monensin (j);

e_{ijkl} = Experimental random error associated to each observation.

Data of CH₄, SCFA, NH₃-N, as well as, rumen protozoa counts were analyzed with the same source of variation described above plus the factor measures repeated in time which refer to different sampling moments, using the mixed model procedure (PROC MIXED) of SAS (Version 9.4, 2015). The model included the effects of oil, monensin, interaction between oil and monensin, time and interaction between oil and time as fixed factors. The effects of period and animal within monensin were included as random factors. For the analysis, 15 different covariance structures were tested, and the one that best fit the statistical model was chosen based on the lowest corrected Akaike information criterion (AICC) (WANG; GOONEWARDENE, 2004). In the presence of oil effect, the comparison of means was performed by Tukey test adjusted to 5% probability.

3.3 RESULTS

3.3.1 Dry matter intake, total tract digestibility and nutrients excretion

Treatment SOY decreased DMI by 11.74% in relation to control treatment, which also led to the reduction of organic matter (OMI), neutral detergent fiber (NDFI) and acid detergent fiber (ADFI) intake in 12.15%, 11.32% and 11.89%, respectively (Table 6). As expected, the consumption of non-fiber carbohydrates (NFCI) was reduced by the addition of lipid sources ($P=0.0003$), while ether extract intake (EEI) was increased ($P=<.0001$) when compared to control treatment.

The addition of monensin in the diet did not alter ($P>0.05$) DM intake or its nutrients, as well as, no additive nor synergistic effect was observed with oils inclusion for these variables.

Table 6 - Effect of diet supplementation with vegetable oils and monensin on dry matter and nutrients intake in Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
DMI, kg/d	9.01	8.89	9.62 ^a	9.06 ^{ab}	8.65 ^{ab}	8.49 ^b	0.2482	0.8791	0.0462	0.4950
CPI, kg/d	1.47	1.45	1.46	1.49	1.46	1.43	0.0412	0.8751	0.7780	0.3925
GEI, Mcal/d	41.1	40.4	41.9	41.1	40.3	39.6	1.1942	0.8773	0.5002	0.3784
NDFI, kg/d	2.97	2.93	3.18 ^a	2.93 ^{ab}	2.87 ^{ab}	2.82 ^b	0.0898	0.8904	0.0360	0.3894
ADFI, kg/d	1.73	1.70	1.85 ^a	1.70 ^{ab}	1.67 ^{ab}	1.63 ^b	0.0563	0.8767	0.0308	0.3886
EEI, kg/d	0.48	0.46	0.25 ^b	0.55 ^a	0.54 ^a	0.52 ^a	0.0267	0.8010	<0.0001	0.5787
NFCI, kg/d	3.57	3.53	4.08 ^a	3.44 ^b	3.36 ^b	3.31 ^b	0.1167	0.9108	0.0003	0.4013
OMI, kg/d	8.38	8.27	8.97 ^a	8.42 ^{ab}	8.03 ^{ab}	7.88 ^b	0.2332	0.8821	0.0407	0.4978

¹DMI: Dry matter intake; CPI: Crude protein intake; GEI: Gross energy intake; NDFI: Neutral detergent fiber intake; ADFI: Acid detergent fiber intake; EEI: Ether extract intake; NFCI: Non-fiber carbohydrates intake; OMI: Organic matter intake; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P<0.05$).

Crude protein digestibility increased ($P=0.0030$) when animals received CAN and SOY treatments (7.8% and 6.1%, respectively) compared to the control treatment (Table 7). Neutral detergent fiber digestibility decreased ($P=0.0222$) in 9.9% and 8.6% with the addition of SUN and SOY diets, respectively. The SOY treatment reduced (18.0%) ADF digestibility in relation to control treatment.

Table 7 - Effect of diet supplementation with vegetable oils and monensin on total tract *in vivo* digestibility of dry matter and its fractions and nutrients excretion in Nellore cattle.

Variables	Monensin		Oil ¹				SEM ²	P-value ³		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
Nutrients digestibility, %										
DM	75.20	75.11	75.18	75.97	74.59	74.89	0.6603	0.9605	0.5990	0.0045
CP	75.96	76.79	72.91 ^b	78.65 ^a	76.57 ^{ab}	77.39 ^a	0.7334	0.7065	0.0030	0.1717
NDF	64.36	64.48	68.44 ^a	65.06 ^{ab}	61.64 ^b	62.55 ^b	1.1206	0.9514	0.0222	0.0635
ADF	54.84	51.92	58.87 ^a	54.21 ^{ab}	52.22 ^{ab}	48.24 ^b	1.6594	0.2239	0.0339	0.1769
EE	82.35	84.77	73.22 ^b	86.44 ^a	86.08 ^a	88.52 ^a	1.4934	0.4304	<0.0001	0.0421
NFC	87.01	86.43	85.72	86.77	87.60	86.79	0.6730	0.7386	0.3527	0.0135
OM	77.06	77.05	77.16	77.76	76.49	76.83	0.6273	0.9968	0.6378	0.0039
TDN	77.17	77.22	74.30 ^b	78.78 ^a	77.61 ^a	78.09 ^a	0.6667	0.9761	0.0018	0.0034
Nutrients excretion, kg/d										
DM	2.24	2.22	2.35	2.16	2.24	2.17	0.0699	0.9498	0.2060	0.0027
CP	0.35	0.33	0.39 ^a	0.32 ^b	0.34 ^{ab}	0.32 ^b	0.0126	0.7117	0.0056	0.0539
N	0.056	0.053	0.062 ^a	0.050 ^b	0.054 ^b	0.052 ^b	0.0020	0.7096	0.0037	0.0436
NDF	1.05	1.03	0.99	1.01	1.09	1.05	0.0382	0.8500	0.4797	0.0474
ADF	0.76	0.78	0.74	0.76	0.78	0.78	0.0276	0.7967	0.8654	0.0221
EE	0.072	0.065	0.066	0.072	0.075	0.063	0.0044	0.6602	0.3116	0.0132
NFC	0.45	0.48	0.57 ^a	0.45 ^b	0.42 ^b	0.43 ^b	0.0241	0.6742	0.0026	0.0184
OM	1.92	1.91	2.01	1.86	1.93	1.86	0.0633	0.9379	0.2647	0.0031

¹CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ²SEM: Standard error of the mean; ³M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P < 0.05$).

There was a significant interaction effect between monensin and oil (MxO) for most of the variables of digestibility and excretion of nutrients, showing that these parameters change due to the combined action of oil sources and monensin in experimental diets. In relation to the observed interaction for the EE digestibility ($P = 0.0421$), when it was decomposed (Figure 1), for the group that did not receive monensin, it was observed that the oils (CAN, SUN and SOY) increased EE digestibility in 22.70%, 21.71% and 21.75%, respectively when compared with control treatment. When monensin was added, it showed an increase in EE digestibility of 13.45% compared to control treatment. In the presence of monensin (combination of oil and monensin), SOY-MON treatment increased the EE digestibility by 24.70%, whereas CAN-MON and SUN-MON treatments did not differ significantly, thus showing an antagonistic type effect, since monensin inhibits the effect of unsaturated oils.

The observed interaction for NDT concentration ($P = 0.0034$) indicated in Figure 2 shows a behavior similar behavior as EE digestibility. Accordingly, the addition of canola oil resulted in increased NDT concentration by 8.47% compared to the control treatment,

whereas SUN and SOY treatments did not significantly differ from these treatments. When monensin was included, an increase of 2.64% of the NDT concentration was observed in relation to control treatment. However, in the presence of monensin combined with the sources of vegetable oils, no significant effect was observed, showing that this is an antagonistic effect.

The increase in crude protein digestibility by the treatments CAN and SOJ was associated with a decrease ($P=0.0056$) of the excretion of this nutrient by these treatments in 17.9%. Oil sources inclusion in experimental diets also decreased the excretion of N ($P=0.0037$) compared to the control treatment (Table 7).

Interaction effect was also observed for the excretion of non-fiber carbohydrates ($P=0.0184$). According to this, SUN treatment promoted a reduction of 41.37% in relation to control treatment, with no difference in any of them to CAN and SOY groups (Figure 3). Monensin (positive control) decreased the NFC excretion by 5.17% compared to control treatment, whereas, when evaluating the combination effect between monensin and oil, it was observed that there is an antagonistic effect and that with the presence of monensin the effect of the oils is lost. From the above, the results of the present study suggest that there is no synergistic or additive effect for digestibility and nutrient excretion variables with monensin and vegetable oils combination.

Figura 1 - Effect of interaction between lipid source and the addition or not of monensin in diets in EE digestibility coefficient.

Source: Romero, 2019

Figure 2 - Effect of interaction between the lipid source and the addition or not of monensin in diet in TDN concentration.

Source: Romero, 2019.

Figure 3 - Effect of interaction between lipid source and the addition or not of monensin in diets in non-fiber carbohydrates excretion.

Source: Romero, 2019.

3.3.2 Ruminal pH

Monensin addition decreased ($P=0.0003$) mean ruminal pH by 4.8%, when compared no monensin addition, and increased by 396.2%; 198.7% and 150.4% the time (min/d) where pH remained below 5.8; 6.0 and 6.2, respectively. The area (h.pH/d) was also increased with the addition of monensin in 152.0%; 209.6% and 174.8%, respectively. There was no significant effect ($P>0.05$) of oil sources inclusion for rumen pH variables evaluated (Table 8). On the other hand, pH values obtained are within the threshold indicative of healthy ruminal conditions, as indicated by Penner & Beauchemin (2010).

Figure 4 shows ruminal pH variation over 24 hours with the association between oil sources and monensin included in diets. The arrows indicate the feeding moment of the animals.

Table 8 - Effect of diet supplementation with vegetable oils and monensin on rumen pH in Nellore cattle.

Variables	Monensin		Oil ¹				SEM ²	P-value ³		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
Daily pH										
Minimum	5.85	5.63	5.81	5.81	5.67	5.70	0.0752	0.1184	0.6732	0.5119
Medium	6.45	6.14	6.30	6.36	6.25	6.27	0.0636	0.0003	0.7145	0.9345
Maximum	6.91	6.62	6.80	6.84	6.70	6.73	0.0508	<0.0001	0.3258	0.5378
Time of pH, min/d										
< 5.8	59.37	294.6	205.0	222.5	104.2	176.3	49.4224	0.0031	0.6737	0.2783
< 6.0	190.6	569.4	377.5	346.3	392.5	403.8	78.2984	0.0005	0.9734	0.8273
< 6.2	320.0	801.3	546.3	441.3	597.5	657.5	97.2856	0.0002	0.5240	0.7195
Area, h.pH/d										
< 5.8	0.25	0.63	0.27	0.63	0.34	0.43	0.1293	0.0370	0.8046	0.2462
< 6.0	0.62	1.92	1.17	1.59	0.93	1.39	0.3093	0.0076	0.7389	0.3225
< 6.2	1.47	4.04	2.70	2.90	2.27	3.14	0.5824	0.0020	0.8515	0.3524

¹CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ²SEM: Standard error of the mean; ³M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil.

Figure 4 - Effect of associate use of monensin and oil sources in diets on mean ruminal pH during 24 hours.

Source: Romero, 2019.

3.3.3 Rumen fermentation products

The SOY treatment decreased ($P=0.0204$) the production (g/kg/d) of acetic acid (30.0%) in relation to control treatment (Table 9). On the other hand, treatments with canola oil (CAN) and soybean oil (SOY) altered ruminal fermentation, reducing the production (g / kg / day) of butyric acid (32.4 and 30.3%, respectively) when compared to the control treatment.

Effect of interaction was observed for the total SCFA ($P=0.0306$), where in the absence of monensin, CAN treatment reduced this variable by 31.52%, when compared to the control treatment, and did not differ from treatments SUN and SOY (Figure 5). However, in the presence of monensin (combination of monensin with canola oil), an antagonistic effect was observed where the effect of SCFA decrease was inhibited. Whereas, in the presence of monensin, soybean oil improved the response, leading to a reduction of 36.97% in total SCFA.

Methane production (g/kg/d) was reduced ($P=0.0423$) in 22.3% in SOY treatment when compared to control treatment (Table 9). Methane emission was also reduced in 17.04% by monensin addition ($P=0.0041$).

Acetate:propionate ratio, when expressed in concentration, decreased ($P=0.0060$) in 18.3% with monensin addition compared to no addition of monensin. However, monensin did not alter ($P>0.05$) the productions (g/kg/d) of acetic, propionic, butyric and total acids. For the variables propionic acid (g/kg/d), acetate:propionate ratio (expressed as production) and REL (%) no effect ($P>0.05$) of oil source and monensin inclusion was observed.

Figure 5 - Effect of interaction between the lipid source and the addition or not of monensin in the production of total short chain fatty acids expressed in g/kg/d.

Source: Romero, 2019.

Table 9 - Effect of diet supplementation with vegetable oils and monensin on short chain fatty acids, methane and relative energy loss in Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
Acetate										
0 min, mmol/L	64.20	67.20	68.90	65.38	65.32	63.20	0.6519	0.0837	0.1450	0.3270
30 min, mmol/L	68.69	71.48	74.27	69.44	69.70	66.93	0.7222	0.1390	0.0653	0.2112
Production, mol/kg/d	2.96	2.76	3.48 ^a	2.65 ^{ab}	2.87 ^{ab}	2.44 ^b	0.1115	0.5479	0.0204	0.0969
Production, g/kg/d	177.5	165.7	208.9 ^a	158.9 ^{ab}	172.3 ^{ab}	146.3 ^b	6.6903	0.5479	0.0204	0.0969
GE, kcal/kg/d	619.4	578.3	728.9 ^a	554.6 ^{ab}	601.3 ^{ab}	510.6 ^b	23.349	0.5479	0.0204	0.0969
Propionate										
0 min, mmol/L	17.57	22.16	21.37	19.23	19.75	19.11	0.4602	0.0010	0.5054	0.3618
30 min, mmol/L	19.57	24.08	23.69	20.87	21.75	20.99	0.5242	0.0053	0.4573	0.2979
Production, mol/kg/d	1.31	1.23	1.46	1.08	1.29	1.24	0.0616	0.6674	0.4099	0.1684
Production, g/kg/d	96.93	90.72	108.1	79.64	95.57	92.05	4.5646	0.6674	0.4099	0.1684
GE, kcal/kg/d	482.5	452.0	539.4	396.6	476.5	456.4	22.732	0.6674	0.4099	0.1684
Butyrate										
0 min, mmol/L	9.12	9.74	10.39	9.13	9.36	8.85	0.1891	0.5818	0.2417	0.5580
30 min, mmol/L	10.37	10.81	11.82	10.10	10.58	9.88	0.2224	0.7442	0.1695	0.4598
Production, mol/kg/d	0.83	0.68	0.93 ^a	0.63 ^b	0.81 ^{ab}	0.65 ^b	0.0313	0.3463	0.0184	0.1908
Production, g/kg/d	72.66	60.20	81.95 ^a	55.36 ^b	71.31 ^{ab}	57.10 ^b	2.7611	0.3463	0.0184	0.1908
GE, kcal/kg/d	433.1	358.8	488.4 ^a	329.9 ^b	425.0 ^{ab}	340.3 ^b	16.456	0.3463	0.0184	0.1908
Total SCFA										
0 min, mmol/L	90.93	98.93	100.7 ^a	93.74 ^{ab}	94.43 ^{ab}	90.86 ^b	1.1509	0.0351	0.0238	0.1219
30 min, mmol/L	98.69	106.1	109.8 ^a	100.4 ^b	102.0 ^{ab}	97.40 ^b	1.2994	0.0681	0.0068	0.0519
Production, mol/kg/d	5.11	4.67	5.88 ^a	4.35 ^b	4.99 ^{ab}	4.33 ^b	0.1818	0.4635	0.0053	0.0306
Production, g/kg/d	348.3	316.1	399.7 ^a	293.9 ^b	340.6 ^{ab}	294.6 ^b	12.282	0.4635	0.0053	0.0306
GE, kcal/kg/d	1524.2	1386.6	1760.1 ^a	1281.1 ^b	1509.6 ^{ab}	1270.8 ^b	54.075	0.4635	0.0053	0.0306
C₂:C₃ ratio										
Concentration	3.87	3.16	3.50	3.60	3.50	3.47	0.0661	0.0060	0.8738	0.3284
Production	2.77	2.46	2.54	2.75	2.67	2.50	0.1374	0.3473	0.9211	0.7956
CH₄										
0 min, mmol/fr	0.018	0.015	0.020 ^a	0.016 ^b	0.017 ^b	0.015 ^b	0.0004	0.0567	0.0002	0.7844
30 min, mmol/fr	0.069	0.058	0.073 ^a	0.061 ^b	0.064 ^{ab}	0.057 ^b	0.0014	0.0006	0.0019	0.9071
Production, mol/kg/d	1.30	1.09	1.32 ^a	1.19 ^{ab}	1.25 ^{ab}	1.03 ^b	0.0357	0.0041	0.0423	0.8381
Production, g/kg/d	20.94	17.37	21.15 ^a	18.99 ^{ab}	20.04 ^{ab}	16.45 ^b	0.5722	0.0041	0.0423	0.8381
GE, kcal/kg/d	275.7	228.6	278.4 ^a	249.9 ^{ab}	263.8 ^{ab}	216.5 ^b	7.5307	0.0041	0.0423	0.8381
REL, %	18.69	17.92	17.94	19.89	17.31	18.09	0.6797	0.7620	0.5238	0.7346

¹CH₄: Methane; REL, %: Relative energy loss; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P < 0.05$).

Figure 6 shows the effect of oil sources on SCFA production (acetic, propionic, butyric and total), CH₄ and REL in relation to control treatment (considered as 100%).

Figure 6 - Effect of oil sources on relative production of short chain fatty acids, methane and relative energy loss.

Source: Romero, 2019.

The concentrations of ruminal $\text{NH}_3\text{-N}$ at 0 and 30 minutes of incubation were increased by 25.6% and 26.3%, respectively, by the addition of monensin in relation to no addition (Table 10). For these variables, no significant effect ($P > 0.05$) of oil source was observed.

Regarding the balance between the concentration at 0 and 30 minutes, there were no significant differences ($P > 0.05$) in oil source or monensin addition.

Table 10 - Effect of diet supplementation with vegetable oils and monensin on the concentration and the balance of ammonia nitrogen of Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
Concentration										
0 min, mg/dL	12.26	16.48	13.16	14.42	15.27	63.20	0.4617	0.0278	0.6067	0.1984
30 min, mg/dL	12.68	17.20	13.42	14.95	16.32	66.93	0.5145	0.0382	0.7270	0.5816
Balance, mg/dL/h	0.843	1.429	0.523	1.051	2.093	0.876	0.2433	0.3004	0.2413	0.9234

¹Balance: Balance per hour between the concentrations at moments 0 and 30 minutes; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; T: Time effect.

3.3.4 Rumen protozoa

SUN and SOY treatments reduced ($P=0.0115$) total protozoa by 44.3% and 55.7%, respectively (Table 11). A similar effect was observed for *Entodinium* (mL), where there was a reduction ($P=0.0154$) of 43.6% and 55.1% for SUN and SOY treatments in relation to control. However, when this genus was expressed as a percentage, an increase was observed with oil sources inclusion ($P=0.0008$).

Table 11 - Effect of diet supplementation with vegetable oils and monensin on total and differential counts of rumen protozoa in Nellore cattle.

Variables	Monensin		Oil ¹				SEM ²	P-value ³			
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO	T
Protozoa, x10 ³ /MI											
<i>Dasytricha</i>	0.42	0.17	0.80	0.15	0.12	0.12	0.0739	0.3985	0.0681	0.5275	0.2822
<i>Isotricha</i>	0.32	0.63	1.71 ^a	0.06 ^b	0.00 ^b	0.12 ^b	0.1021	0.3403	0.0019	0.8724	0.4816
<i>Entodinium</i>	481.6	438.3	695.5 ^a	440.1 ^{ab}	392.2 ^b	311.9 ^b	22.589	0.5671	0.0154	0.0955	<0.0001
<i>Diplodiniinae</i>	4.63	4.25	10.47 ^a	2.79 ^b	2.46 ^b	2.04 ^b	0.4413	0.7747	<0.0001	0.2817	0.0030
Total	487.1	443.4	708.9 ^a	443.1 ^{ab}	394.8 ^b	314.2 ^b	22.866	0.5628	0.0115	0.0866	<0.0001
Protozoa, %											
<i>Dasytricha</i>	0.116	0.069	0.261	0.033	0.040	0.037	0.0278	0.3687	0.1191	0.8381	0.3012
<i>Isotricha</i>	0.056	0.088	0.252 ^a	0.008 ^b	0.000 ^b	0.030 ^b	0.0146	0.3907	0.0017	0.9432	0.1524
<i>Entodinium</i>	99.05	99.03	98.24 ^b	99.30 ^a	99.36 ^a	99.26 ^a	0.0695	0.8982	0.0008	0.1396	0.0096
<i>Diplodiniinae</i>	0.78	0.77	1.18 ^a	0.66 ^b	0.60 ^b	0.65 ^b	0.0539	0.9579	0.0202	0.0242	0.0193

¹CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ²SEM: Standard error of the mean; ³M: Monensin effect; O: Oil effect; T: Time effect; MxO: Interaction between monensin and oil;

^{ab}Different letters in the same line statistically differ by Tukey test ($P<0.05$).

On the other hand, the addition of canola (CAN), sunflower (SUN) and soybean (SOY) oils in cattle diets reduced *Isotricha* (96.5%, 100% and 93.0%, respectively) and of the subfamily *Diplodiniinae* (73.3%, 76.5% and 80.5%) in relation to control diet, showing a defaunating effect. Similar behavior was observed when the protozoa from the genus *Isotricha* were expressed in percentage, with a decrease of 96.8%, 100% and 88.1%, in the CAN, SUN and SOY treatments, respectively, when compared to control treatment. For the subfamily *Diplodiniinae*, when expressed as a percentage, interaction effect ($P=0.0242$) was observed between MxO (Figure 7). According to this, the figure shows that with the addition of the lipid sources in the form of oil, there was a reduction of the protozoa of this subfamily in 72.60% and 70.54% in SUN and SOY treatments, respectively, in relation to the control treatment. The CAN treatment did not differ from the others. The inclusion of monensin generated a reduction of 43.83%, in comparison to the control. In the presence of

monensin combined with the vegetable oils, it was observed that the lipid sources lose the effect of reducing the protozoa of this subfamily, showing an antagonistic effect for SUN-MON and SOY-MON treatments, with a numerical reduction of 45.89% and 40.41%, respectively, when compared to the control treatment.

No significant effect ($P>0.05$) of oil source or monensin was observed for *Dasytricha* protozoa when expressed in mL or percentage. There was no effect of monensin for total or differential count of protozoa.

Figure 7 - Effect of interaction between lipid source and the addition or not of monensin on concentration (%) of Diplodininae protozoa.

Source: Romero, 2019.

3.3.5 Energy partition

No effect ($P>0.05$) of lipid source, nor monensin, as well as, interaction was observed for energy consumption (Mcal/animal/d). For energy released in the rumen in the form of butyric acid in relation to the GE consumed (Table 12), CAN and SOY reduced this variable by 27.9% and 36.5%, respectively. Similar effect was observed for energy released in the rumen in the form of butyric acid expressed in percentage of DE, where there was a decrease of 28.9% (CAN) and 37.3% (SOY). However, monensin inclusion did not affect any of these variables.

Table 12 - Effect of diet supplementation with vegetable oils and monensin on energy release estimate in gastrointestinal tract of Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
GEI, Mcal/animal/d	41.1	40.4	41.9	41.1	40.3	39.6	1.1944	0.8772	0.5007	0.3777
Energy release in the rumen										
Acetate										
Mcal/animal/d	3.15	3.39	3.84	2.99	3.38	2.88	0.2119	0.7461	0.0756	0.0334
GE, %	7.85	7.89	8.62	7.22	8.40	7.24	0.4535	0.9709	0.4258	0.0511
DE, %	10.3	10.4	11.4	9.43	11.0	9.48	0.5957	0.9319	0.4130	0.0401
Propionate										
Mcal/animal/d	2.47	2.65	2.94	2.15	2.61	2.55	0.2114	0.7948	0.3161	0.0641
GE, %	5.88	6.49	6.99	5.17	6.47	6.10	0.4332	0.6020	0.3820	0.1643
DE, %	7.72	8.58	9.26	6.80	8.53	8.00	0.5812	0.5871	0.3925	0.1652
Butyrate										
Mcal/animal/d	2.21	2.17	2.53	1.79	2.36	2.06	0.1618	0.9375	0.1112	0.4465
GE, %	5.14	5.01	6.03 ^a	4.35 ^b	6.07 ^a	3.83 ^b	0.3897	0.9045	0.0032	0.1652
DE, %	6.77	6.58	7.99 ^a	5.69 ^b	8.02 ^a	5.01 ^b	0.5262	0.8971	0.0035	0.1295
Total SCFA										
Mcal/animal/d	7.84	8.21	9.31	6.94	8.35	7.49	0.5094	0.8435	0.0562	0.0411
GE, %	19.2	20.1	21.6	16.7	20.9	19.2	1.0482	0.7613	0.1973	0.2050
DE, %	25.1	26.5	28.6	21.9	27.6	25.1	1.3921	0.7228	0.2056	0.1769
Methane										
Mcal/animal/d	1.42	1.32	1.45	1.33	1.39	1.31	0.0783	0.7522	0.7190	0.9982
GE, %	3.46	3.30	3.52	3.24	3.53	3.23	0.1850	0.7192	0.6746	0.9942
DE, %	4.56	4.37	4.70	4.23	4.65	4.27	0.2586	0.7206	0.6026	0.8817
Energy release in the intestine										
Mcal/animal/d	20.8	20.0	19.9	22.0	19.6	20.1	0.8520	0.6588	0.4325	0.3184
GE, %	50.7	49.6	47.2	53.9	48.2	51.5	1.3430	0.6919	0.0962	0.0516
DE, %	66.4	64.9	62.1	69.4	63.7	67.6	1.5183	0.7111	0.1117	0.1834
Energy release in the feces										
Mcal/animal/d	9.59	9.57	9.95	9.36	9.70	9.33	0.3065	0.9797	0.4410	0.0026
GE, %	23.7	23.8	24.1	22.9	24.2	23.7	0.6634	0.9372	0.6327	0.0045

¹GEI: Gross energy intake; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P<0.05$).

There was no significant effect ($P>0.05$) of lipid source or monensin inclusion for the other variables of energy released in the rumen, in the intestine or through feces. However, an interaction effect when monensin and oil (MxO) were combined for acetic acid when expressed as Mcal/ animal/d ($P=0.0334$) and in relation to DE consumed ($P=0.0401$) was observed as indicated in Figures 8 and 9, respectively. For the observed interaction in the energy released in the rumen as acetic acid (Mcal/ani/day), when vegetable oils were added to animals' diets, there was no significant difference for this

variable (Figure 8). On the other hand, the addition of monensin generated an increase in acetic acid release (Mcal / animal per d) of 28.41%, in relation to control treatment. However, when the combination of monensin with the oils was evaluated, a reduction of the acetic acid release was observed in SOY-MON treatment when compared to control treatment, without difference from CAN-MON and SUN-MON treatments.

Lipids inclusion in diets did not affect the energy released as acetic acid expressed in percentage of DE, in the absence of monensin (Figure 9). On the other hand, with the addition of monensin, there was a 15.91% increase in energy release as acetic acid (% ED) in relation to the control treatment. In the presence of monensin, there is a potentialized effect (synergism), in the reduction of this variable, with the association of monensin and soybean oil (37.07%).

Figure 8 - Effect of interaction between the lipid source and the addition or not of monensin on acetic acid production (Mcal/animal/d).

Source: Romero, 2019.

Interaction between lipid source and monensin inclusion ($P=0.0411$) was also observed for total SCFA expressed as Mcal/animal/d (Figure 10). For the group with oil addition (absence of monensin), no significant effect was observed. On the other hand, when monensin was added, total ACGG was increased (26.68%) in relation to control. With the combination of monensin and oil, there was a reduction of 22.92% in SOY-MON treatment compared to control, showing an antagonistic effect for this variable.

Figure 9. Effect of interaction between the lipid source and the addition or not of monensin on acetic acid production in relation of percentage digestible energy.

Source: Romero, 2019.

Figure 10. Effect of interaction between the lipid source and the addition or not of monensin on total short chain fatty acids production (Mcal/animal/d).

Source: Romero, 2019.

3.3.6 Nitrogen compounds, microbial protein synthesis and nitrogen balance

Lipid sources decreased ($P=0.0129$) uric acid concentration in urine in 20.5% (CAN), 22.5% (SUN), and 21.2% (SOY) (Table 13). For the percentage of allantoin of purine derivatives (AI% PD), in SOY treatment ($P=0.0195$) its concentration was 2.99% higher compared to control treatment (CONT-WMON). Effect of monensin addition in the diet ($P=0.0493$) was observed for this variable with an increase of more than 3.60% in relation to the treatment without monensin addition.

Urinary volume (L) and variables such as allantoin (mmol/L), purine derivatives (mmol/d), microbial nitrogen (g/d) and microbial protein synthesis efficiency (g Pmic/kg RDP) were not affected ($P>0.05$) neither by the monensin factor nor by the oil factor.

Table 13 - Effect of diet supplementation with vegetable oils and monensin on nitrogen compounds excretion, microbial nitrogen synthesis and efficiency of microbial protein synthesis in Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
Urinary volume, L	8.27	9.21	8.35	9.05	8.54	9.03	0.4750	0.5339	0.9020	0.8976
Nitrogen compounds										
Al, mmol/L	12.25	9.45	11.47	10.80	10.01	11.12	0.5924	0.1374	0.4713	0.2081
UA, mmol/L	1.943	1.115	1.822 ^a	1.448 ^b	1.411 ^b	1.435 ^b	0.1264	0.0834	0.0129	0.3485
Al, mmol/d	89.17	84.23	78.98	91.62	82.17	94.03	4.1914	0.7097	0.2002	0.1308
UA, mmol/d	15.49	9.99	15.30	12.23	11.27	12.16	1.0115	0.0864	0.2960	0.8533
Total PD, mmol/d	103.4	94.20	91.71	103.9	93.45	106.2	4.8059	0.5437	0.2721	0.1315
Al (%) PD	86.31	89.54	86.39 ^b	88.31 ^{ab}	87.96 ^{ab}	89.06 ^a	0.5482	0.0493	0.0195	0.7620
Pabs, mmol/d	74.71	63.51	61.22	74.87	62.74	77.61	5.1220	0.4374	0.2952	0.1436
Synthesis of N microbial										
N mic, g/d	54.32	46.18	44.51	54.44	45.62	56.43	3.7241	0.4374	0.2952	0.1435
N mic, mg/kg BW	118.0	92.36	93.28	110.9	93.22	123.4	9.5429	0.3261	0.3954	0.1712
Microbial protein synthesis										
gN/kg of OM	11.69	9.181	10.53	10.64	9.244	11.32	0.7418	0.2074	0.5968	0.4620
gPmic/kg of RDP	448.3	373.1	351.0	423.8	409.8	458.1	27.158	0.2872	0.4900	0.3557

¹AL: Allantoin; UA: Uric acid; PD: Purines derivatives; AL (%) PD: Percentage of allantoin of purines derivatives; Pabs: Absorbed purines; N mic: Microbial nitrogen; RDP: ; OM: Organic matter; RDP: Rumen degradable protein; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P<0.05$).

No significant effect ($P>0.05$) of oil source or monensin for N ingested (g/d) was observed. However, in relation to nitrogen excreted (g) on feces, it was reduced ($P=0.0038$) when CAN and SOY oils were added in experimental diets in 18.7% and 17.0%, respectively (Table 14). The opposite effect was observed for N excreted via urine, as it increased in SOY treatment in 20.2% when compared to control treatment. These results were closely related to that observed for nitrogen balance, and N in the feces (g/kg of ingested N) which were reduced ($P=0.0030$) in treatments CAN (21.2%) and SOY (16.6%). Contrary from what was observed for N in urine, which was increased ($P=0.0333$) by 23.3% in treatment SOY, in relation to control treatment.

There was no significant effect ($P>0.05$) of monensin for any of the evaluated variables.

Table 14 - Effect of diet supplementation with vegetable oils and monensin on nitrogen balance in Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CON	CAN	SUN	SOY		M	O	M x O
N ingested, g/d	235.3	231.6	233.5	238.3	232.9	229.1	6.6035	0.8762	0.7832	0.3887
N excreted, g/d										
Feces	56.03	53.50	62.25 ^a	50.62 ^b	54.53 ^{ab}	51.67 ^b	2.0175	0.7088	0.0038	0.0440
Urine	92.19	94.21	80.34 ^b	93.22 ^{ab}	98.51 ^{ab}	100.7 ^a	4.5031	0.9011	0.0454	0.2069
Total	148.2	147.7	142.6	143.9	153.0	152.4	4.9934	0.9790	0.3218	0.0545
Nitrogen retention										
N consumed, %	36.34	35.99	38.63	39.60	33.74	32.71	1.7754	0.9344	0.1886	0.2159
N absorbed, %	47.79	47.05	52.86	50.45	44.19	41.99	2.2756	0.9067	0.0937	0.2865
N retained, g/d	87.11	83.84	90.94	94.43	79.81	76.73	5.2135	0.8069	0.2472	0.2635
N balance, g/kg of N ingested										
Feces	240.4	232.1	270.9 ^a	213.5 ^b	234.3 ^{ab}	226.0 ^b	7.3355	0.7064	0.0030	0.1714
Urine	396.2	407.9	342.8 ^b	390.4 ^{ab}	428.2 ^{ab}	446.8 ^a	17.999	0.8030	0.0333	0.3102
Retained	363.4	359.9	386.3	396.0	337.4	327.1	17.753	0.9344	0.1886	0.2159

¹N: Nitrogen; N retained: N ingested – N excreted (feces + urine); ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P < 0.05$).

3.4 DISCUSSION

The inclusion of polyunsaturated fatty acids (PUFA) rich in linolenic acid (n-3) in cattle diets, such as soybean oil, may increase cholecystokinin secretion (CCK) and inhibit reticulum-ruminal motility, resulting in decreased dry matter intake (DMI) (NICHOLSON; OMER, 1983). Harvatine & Allen (2005) indicated that the reduction in DMI may be related to a satiety effect and a decrease in digestive motility, which results in changes in ruminal fermentation due to reduced fiber digestibility in the rumen and consequent higher rumen fill (JENKINS; McGUIRE, 2006).

Weiss & Pinos-Rodriguez (2009), Hess et al. (2008) and Zinn et al. (2000) reported that levels greater than 50 and 60 g of lipids/kg of DM affect DMI, whereas, NRC (2001), UEDA et al. (2003), Harvatine & Allen (2006) reported that up to a maximum of 30 g of lipids in the form of oil per kg of DM can be used in cattle diets, since a level higher than this has minimal negative effects on microbial growth and ruminal function. In the present experiment, a level of 35 g of lipids in the form of oil per kg of DM was included in cattle diets.

The reduction observed in non-fiber carbohydrates intake and the increase in ether extract intake, when a source rich in ω -3 polyunsaturated fatty acids (PUFA) was included in cattle diets, can be associated to the partial substitution of non-structural

carbohydrates by lipids in total DM. The increase in the digestibility of ether extract (D_{EE}) in CAN, SUN and SOY treatments was explained due to the increase in lipid content of diets, as well as, their degree of unsaturation, which improves fatty acids digestibility (JENKINS et al., 2008; DUCKETT; GILLIS, 2010).

Greater digestibility of crude protein (D_{CP}) observed when monounsaturated fatty acids (MUFA) (canola) and PUFA (soybean) sources are included in diets is probably due to protozoa counts reduction subsequent to the lipid inclusion, which causes an increase in bacterial biomass and, therefore, a globally larger proteolytic activity (USHIDA et al., 1991). Greater D_{CP} was also related to a reduction in CP and N excretion observed in these treatments. Beauchemin et al. (2007) show that greater protein digestibility when lipid sources are included in diets in the form of oils would be an additional benefit for livestock, since this type of supplementation could reduce feed protein cost.

The reduction in digestibility of NDF (D_{NDF}) and ADF (D_{ADF}) observed with PUFAs in SUN and SOY treatments may be due a decrease in cellulolytic population and the coating effect of lipids in feed particles, which affects ruminal fermentation, reducing fiber degradability and digestibility. The addition of lipids in cattle diets may result in a reduction of 50% or more in structural carbohydrates digestion in the rumen (IKWUEGBU; SUTTON, 1982; JENKINS; PALMQUIST, 1984). This reduction in digestion is followed by a decrease in methane (CH_4) production, hydrogen ions, and short chain fatty acids (SCFA), including acetate:propionate ratio (BOGGS et al., 1987; CZERKAWSKI; CLAPPERTON, 1984; IKWUEGBU; SUTTON, 1982). These effects were observed in the present experiment. When lipids decrease ruminal fermentation, fermentation in the large intestine is limited, which can affect fiber digestibility throughout the gastrointestinal tract (BOGGS et al., 1987; JENKINS, 1988). Deleterious effect in fiber digestibility was observed by McGinn et al. (2004) when 4.5% (DM basis) of sunflower oil was included in cattle diet. A reduction of 33.0% in D_{NDF} and of 29.0% in D_{ADF} was reported.

Greater total digestible nutrients (TDN) concentration in the diets with lipid sources inclusion without monensin was due to the higher energetic density. However, the present experiment showed that there is an antagonistic effect when there is a combination between monensin and unsaturated lipid sources, because monensin inhibits the effect of oils and tend to decrease TDN concentration.

The inclusion of 300 mg/animal/d of sodium monensin in diets (equivalent to 32 mg/ kg DM) had no effect on DMI. Also, studies that evaluated the inclusion dose of 22

(PLAIZIER et. al., 2000; OSBORNE et. al., 2004) or 24 mg of monensin/kg of DM (BELL et. al., 2006) observed no effect on DMI.

In the present study, no effect of monensin supplementation was also observed for total tract apparent digestibility or excretion of nutrients. It has been reported that the influence of this ionophore in these variables are not consistent. Monensin supplementation reduced ruminal digestibility of nutrients in several studies with cattle (OWENS et al., 1978; SIMPSON, 1980; MUNTIFERING et al., 1981), but not in others (MORRIS et al., 1990; ROGERS et al., 1991). Osborne et al. (2004) did not observe effect of monensin supplementation on fiber ruminal digestibility. Likewise, the authors indicate that DM, CP, EE and GE were not affected by monensin inclusion in the diet. In contrast, Haïmoud et al. (1995) observed that monensin supplementation altered nutrients digestibility, reducing ruminal fiber digestibility. However, post ruminal digestibility (small intestine) of fiber was greater with monensin supplementation. According to the above, the reasons for these discrepancies between the studies may be related to differences in the levels of inclusion of monensin in the diet and the interactions between intake and feed composition (MUTSVANGWA et al., 2002).

The reduction of ruminal pH with monensin supplementation observed in the present experiment was not expected to occur, as monensin favors the ruminal environment by inhibiting lactate production, which results in high ruminal pH (RUSSELL; HOULIHAN, 2003).

The inclusion of lipids source in diets had no significant effect on ruminal pH. Normally, the inclusion of lipids in the form of oil in cattle diet is associated to a decrease in ruminal pH. However, the observed effect seems to be associated to an antagonistic effect of monensin, which did not allow lipids sources to show their effect of reduction of ruminal pH. In this sense, monensin is used in this type of diets, to attenuate and avoid ruminal acute acidosis in beef (COOPER; KLOPFENSTEIN, 1996; RUSSELL; HOULIHAN, 2003) and dairy cattle (GREEN et al., 1999), as monensin inhibits lactate production and allows the proliferation of cellulolytic bacteria, avoiding some metabolic disorder, mainly acidosis (KRAJCARSKI-HUNT et al., 2002; PLAIZIER et al., 2001).

The reduction of acetic acid production (30.0%) generated by a source of ω -3 PUFA (soybean) was directly associated to CH₄ decrease (22.2%). Methane reduction was also associated to PUFAs action that reduced DMI and fiber digestibility. It has been reported that lower feed intake contributes to decreased CH₄ production (KNAPP et al., 2014). In addition, the reduction in CH₄ production can also be explained by the decrease

in butyric acid and total SCFA observed by the addition of MUFA (canola) and ω -3 PUFA (soybean). The reduction of butyric acid, observed in the present experiment, can be because of defaunation (WILLIAMS; COLEMAN, 1997) generated by lipids, mainly Holotrich protozoa (*Isotricha* and *Diplodininae*). It is worth to mention that the efficacy of lipid inclusion in diets in reducing CH₄ emissions depends on the level of supplementation, lipid source and type of inclusion, for example, refined oil or oilseeds, as well as, the type of diet (BEAUCHEMIN et al. 2008). Despite the possibility of CH₄ reduction of more than 40% when high levels of lipids are included (MACHMULLER; KREUZER, 1999; JORDAN et al., 2006b), a reduction of 10 to 25% is more likely to be achieved (BEAUCHEMIN et al., 2008). Omega-3 (n-3) and omega-6 (n-6) fatty acids decreased CH₄ release by up to 20% (LOMAS, 2013). While lauric (C12:0), myristic (C14:0) and linoleic (C18:2) acids decreased CH₄ release, due to the observed reduction in the number of methanogenic microorganisms. In addition, lauric (C12:0) and linoleic (C18:2) acids negatively affected protozoa population (DOHME et al., 2001).

The addition of lipid sources, mainly in the form of oil, suppress enteric production of CH₄, due to reduction in DMI and nutrient digestibility (BEAUCHEMIN et al., 2007), and a direct toxic effect of unsaturated fatty acids on methanogenic population (ALLEN 2000; BOADI et al., 2004; BEAUCHEMIN et al., 2009a). These effects were observed in the present experiment.

For ruminal fermentation products, different sources of oil included in cattle diets did not affect propionic acid production. This result was related to observed ruminal pH and relative energy loss (REL), which were also not affected by lipid sources inclusion in diets. Monensin also had no effect on acetic, propionic, butyric and total SCFA production. However, in the present experiment, acetate:propionate (A:P) ratio, when expressed as concentration, was decreased with monensin supplementation. Among the effects of monensin on ruminal fermentation, studies indicate that this ionophore decreases lactic acid production *in vitro* (DENNIS et al., 1981), as well as *in vivo*. In cattle supplemented with monensin, reduced lactate concentrations, as well as, high ruminal pH values have been described (NAGARAJA et al., 1982). This effect was not observed in the present experiment. Other studies show that total SCFA production is not affected, but that there is a decrease in the A:P ratio, since propionate-producing and lactate-utilizing bacteria are favored, and the acetate, butyrate, lactate and ammonia-producing bacteria are less favored (RUSSELL; STROBEL, 1989; HRISTOV et al., 2001; GUAN et al., 2006). This observation was described in the present study. Therefore, consistent with other studies,

monensin supplementation decreased ruminal A:P ratio (BROWN; HOGUE, 1985; TOMKINS et al., 2015; WANG et al., 2015), but no effect of monensin supplementation was observed on butyric acid proportion (GEHMAN et al., 2008; TOMKINS et al., 2015; VENDRAMINI et al., 2015).

The reduction in CH₄ emissions obtained with vegetable oils supplementation was due to their direct inhibition of methanogenic microbiota in the rumen. This may occur, as vegetable oils can suppress protozoa growth, as observed in this experiment, where sources of PUFA (sunflower and soybean oils) reduced total protozoa and ciliates of the genus *Entodinium*. The addition of lipid sources (MUFA and PUFA) in the form of oil in diets shows a potential defaunation effect on Holotrich protozoa population, such as *Isotricha* and the subfamily *Diplodininae*, which are responsible for great part of CH₄ production. Previous studies showed that Holotrich protozoa had greater number of endosymbiotic methanogenic microorganisms in relation to Entodiniomorph protozoa (BELANCHE et al., 2014), possibly because Holotrich protozoa have more active hydrogenosomes than Entodiniomorph (PAUL et al., 1990). These differences may explain the greater impact of Holotrich protozoa in ruminal methanogenesis process in comparison to Entodiniomorph (BELANCHE et al., 2015).

Moss et al. (2000) indicated that unsaturated lipid sources, such as soybean oil, exhibit defaunating properties and interfere with Gram-positive bacteria activity. As previously mentioned, there is a relationship between protozoa concentration and CH₄ emissions (GUYADER et al., 2014). It is estimated that between 9 and 37% of rumen CH₄ production can be attributed to protozoan-associated methanogenic microorganisms (FINLAY et al., 1994; NEWBOLD et al., 1995; MACHMÜLLER et al., 2003). The effect of defaunation on CH₄ production showed that protozoa removal from the rumen results in 13% reduction in this gas production (HEGARTY, 1999). Morgavi et al. (2010) indicated that rumen defaunation resulted in 10.5% reduction in CH₄ emissions. It is evident that the methanogenesis process is directly affected by the presence of protozoa in the rumen (NEWBOLD et al., 1995). Due to the symbiotic relationship between methanogenic and protozoa population (FINLAY et al., 1994), methane release can be reduced by 20 to 30% by means of defaunation of protozoa in the rumen (KREUZER et al., 1986). *In vitro* and *in vivo* experiments show that protozoa are sensitive to unsaturated fatty acids C18:3, C18:2 and C18:1, in this order of influence (MACHMÜLLER et al., 1998; HRISTOV et al., 2004). These fatty acids are present in the lipid sources used in this research. Therefore, the

results obtained allow us to conclude that supplementation with vegetable oils interferes on protozoa metabolic activity.

For differential protozoa counts, predominance of ciliates of the genus *Entodinium* (%) was observed, mainly when lipid sources rich in MUFA and PUFA were included in diets. This result is in agreement with the observations of other authors, who evaluated different feeding managements on protozoa counts in cattle, such as diets rich in sugarcane (FRANZOLIN; FRANZOLIN, 2000), diets rich in concentrate, with or without lipids addition (TOWNE et al., 1990), or the use of ionophores in diets rich in forage or concentrate (GUAN et al., 2006). Ikwuegbu & Sutton (1982), Towne et al. (1990) and Ivan et al. (2001) evaluated the inclusion of lipids in ruminant diets and verified decrease and even total disappearance of protozoa, with *Entodinium* being the least affected. The present study indicates that there is an antagonistic effect with the combination between monensin and polyunsaturated oil sources on the subfamily *Diplodininae*, when expressed as percentage. This is due to the fact that the combination of monensin with lipids inhibits the defaunation action of PUFA sources on these protozoa population.

In this research, monensin had no effect on protozoa population evaluated, which is related to the absence of monensin effect on digestibility and nutrient excretion. Monensin presented contradictory results on ciliates population in the rumen in relation to its efficacy. Short-term studies demonstrated a reduction in the number of protozoa when monensin was added to cattle diets. However, an increase in protozoa counts has been observed after three weeks of monensin supplementation. Guan et al. (2006) evaluated monensin combined or not with lasalocid showing that the effect of ionophores is transient and that ciliates can create resistance to ionophores, when used for longer periods. Rogers et al. (1997) showed that the negative effect of monensin on protozoa decreased over time in animals fed high concentrate diets and the intensity of this effect was dependent on type of protozoa. Several studies also reported the resistance of Gram-positive bacteria to monensin (NEWBOLD et al., 1993; RAVVA et al., 2013; KIM et al., 2014).

The concentration of ruminal $\text{NH}_3\text{-N}$ was higher with monensin supplementation at time 0 (25.6%) and 30 minutes (26.3%) of incubation compared with no monensin supplementation. This fact can be associated to an inefficient effect of protein utilisation by ruminants. It was expected a reduction in $\text{NH}_3\text{-N}$ concentration with monensin inclusion in cattle diets, as proteolytic and amino acids fermenting bacteria are sensitive to ionophores (GOMES, 2009). Also, the higher concentration of ammonia with monensin supplementation may be due to lower use of nitrogen for bacterial growth (EIFERT et al.,

2005). This effect explains the absence of significant effect on microbial protein synthesis (g/d or mg/kg of BW) and on efficiency of synthesis (g/kg of OM or g P_{mic} /kg of RDP) by monensin. It was also expected that ruminal NH_3 -N concentration would decrease with protozoa counts reduction caused by vegetable oil supplementation, but this did not occur. This effect was expected, because one of the main characteristics of the defaunation is the reduction of NH_3 -N concentration in the rumen, due to the reduction of the predatory and proteolytic activity of protozoa (DOREAU; FERLAY, 1995). From the above, the absence of lipid source effect on NH_3 -N was related to the non-significant effect on microbial protein efficiency and synthesis. Studies indicate that lipid supplementation generally does not result in changes in NH_3 -N concentration in the rumen (DOREAU; FERLAY, 1995). In general, nitrogen recycling is probably not modified by lipids in most experiments and there is no reason for a variation on amino acid catabolism when lipid sources are added (ELMEDDAH et al., 1994). On the other hand, the variations in the NH_3 -N concentration depend mainly on ruminal pH, reason why no lipid source effect was observed on NH_3 -N concentration, since values of ruminal pH remained unchanged.

Lipid supplementation did not modify the passage rate and the turnover of ruminal fluid, thus, the outflow of ammonia through the reticulum-omasal orifice was not altered. This effect could have occurred in the present experiment, reason why no significant difference was observed for NH_3 -N. This effect was directly related to the absence of significant effect of treatments on microbial protein synthesis (g/d or mg/kg of BW) and on efficiency of synthesis (g/kg of OM or g P_{mic} /kg of RDP), which in turn, was related to the absence of significant effect on OM digestibility and on propionic acid production.

Doreau & Ferlay (1995) indicate that unsaturated lipid sources have little influence on microbial protein synthesis. However, an increase in the efficiency of microbial synthesis can be observed more frequently, when there is a reduction in protozoa number or in OM digestibility. Eifert et al. (2005) reported that microbial synthesis was reduced by soybean oil and monensin inclusion in diets, without differences for the efficiency of microbial synthesis (in g N/kg of rumen degradable organic matter), which was justified by inhibitory effects of treatments on bacterial growth.

Partial rumen defaunation has been proposed to increase the efficiency of microbial protein synthesis and to decrease N losses in urine (KOENIG et al., 2000; HRISTOV; JOUANY, 2005). However, the use of vegetable oils as a defaunating agent also decreases ruminal digestion of DM and NDF, limiting ruminal microbial protein synthesis (FIRKINS et al., 2007), an effect observed in the present experiment.

From the data obtained with the ex-situ technique, it was possible to calculate the daily production of ruminal fermentation products, as well as the energy released along the gastrointestinal tract. These data become of great contribution to science due to the low availability of similar data in the literature.

The lack of effect on gross energy intake by both vegetable oils and monensin supplementation is explained by the fact that the diets evaluated were isoenergetic. The absence of effect on the energy released in the rumen, intestine and feces in the form of acetic, propionic and total SCFA, can be explained by the absence of effect on REL.

Buddle et al. (2011) indicate that 5 to 9% of gross energy (GE) of ingested diet is lost as methane. These data were consistent to what was observed in the present study.

3.5 CONCLUSION

A source of omega-3 polyunsaturated fatty acid, such as soybean oil results in greater efficiency of ruminal fermentation process modification, since it reduces acetic acid and, consequently, methane production. The reduction of total protozoa in the rumen had a direct effect on CH₄ emission decrease. Sources of MUFA and PUFA decrease butyric acid production and total SCFA, as well as, result in a defaunating effect of Holotrich protozoa (*Isotricha* and *Diplodininae*).

The present study demonstrates that the combination of monensin with a source of omega-3 polyunsaturated fatty acid, such as soybean oil, results in a potentializing effect (synergism) on the reduction of total short chain fatty acids (g / kg / d) and the release of energy in the form of acetic acid expressed in percentage of DE. On the other hand, the combination of monensin and vegetable oils (unsaturated fatty acids) resulted in an antagonistic effect on variables that were increased (EE digestibility and TDN concentration) or reduced (excretion of non-fiber carbohydrates, total SCFA, protozoa counts of the subfamily *Diplodininae*) with the addition of vegetable oils. This indicates that in the presence of monensin, a contrary effect to that observed with vegetable oils inclusion was obtained or the significant effect generated by the oils was inhibited.

Lipid sources inclusion in diets result in increased crude protein digestibility, which leads to lower concentration of uric acid in the urine. However, the retention of N and the efficiency of Pmic synthesis were not affected by the treatments, showing no associative or additive effect between monensin and vegetable oil.

REFERENCES

ALLEN, M.S. Effects of diet on short-term regulation of feed intake by lactating dairy cattle, **Journal of Dairy Science**, v.83, n.7, p.1598-1624, 2000.

AOAC International, 2000. **Official Methods of Analysis of AOAC International**. 17th Edition. Gaithersburg, MD (USA).

AOCS. AMERICAN OIL CHEMISTS SOCIETY. **Official Method Am 5-04, Rapid determination of oil/fat utilizing high temperature solvent extraction**. 5 ed., The American Oil Chemists Society, Urbana, IL, USA, 2005.

BEAUCHEMIN, K.A.; MCGINN, S.M. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil, **Journal of Animal Science**, v. 84. p.1489-1496, 2006.

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

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.

BEAUCHEMIN, K.A.; MCGINN, S.M.; BENCHAAAR, 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. 2118-2127, 2009.

BEAUCHEMIN, K. A.; H.H. JANZEN, S.M. LITTLE, T.A. MCALLISTER, S.M. MCGINN. Life cycle assessment of greenhouse gas emissions from beef production in western Canada: A case study. **Agricultural Systems - Journal**. v.103, p 371-379, 2010.

BELANCHE, A.; DE LA FUENTE, G.; NEWBOLD C. J. Study of methanogen communities associated with different rumen protozoal populations. **FEMS Microbiology Ecology**, v. 90, p.663–677, 2014.

BELANCHE A.; DE LA FUENTE G.; NEWBOLD C. J. Effect of progressive inoculation of fauna-free sheep with holotrich protozoa and total-fauna on rumen fermentation, microbial diversity and methane emissions. **FEMS Microbiology Ecology**, v.362, p.1–10, 2015.

BELL, J.A.; GRIINARI, J.M.; KENNELLY, J.J. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. **Journal of Dairy Science**, v.89, p.733–748, 2006.

BOADI, D.; BENCHAAAR, C.; CHIQUETTE, J.; MASSÉ, D. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. **Canadian Journal of Animal Science**, v.830, p.319-335, 2004.

BOGGS, D.L.; BERGEN, W.G.; HAWKINS, D.R. Effects of tallow supplementation and protein withdrawal on ruminal fermentation, microbial synthesis and site of digestion. **Journal of Animal Science**, v. 64, p. 970, 1987.

BROWN, D.L.; HOGUE, D.E. Effects of feeding monensin sodium to lactating goats: Milk composition and ruminal volatile fatty acids. **Journal of Dairy Science**, v.68, p.1141-1147, 1985.

CHEN, X.B.; GOMES, M.J. **Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives** – an overview of technical details, INTERNATIONAL FEED RESEARCH UNIT, Bucksburn, Aberdeen: Research Institute, 1992, 21p, (Occasional publication).

CHIZZOTTI, M.L.; VALADARES FILHO, S.C.; VALADARES, R.F.D. et al. Excreção de creatinina em novilhos e novilhas. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 41., 2004, Campo Grande. **Anais...** Campo Grande: Sociedade Brasileira de Zootecnia, 2004. CD-ROM. Nutrição de ruminantes.

COOPER, R.; KLOPFENSTEIN, T. Effect of Rumensin and feed intake variation on ruminal pH. In: **Scientific Update on Rumensin/Tylan/Mycotil** for the Professional Feedlot Consultant. Elanco Animal Health, Indianapolis, IN. pp A1–A14, 1996.

CZERKAWSKI, J.W.; CLAPPERTON, J.L. Fats as energy-yielding compounds in the ruminant diet, Page 249 in Fats in **Animal Nutrition**, J, Wiseman, cd, Butterworths, Boston, MA, 1984.

DEHORITY, B.A. **Laboratory manual for classification and morphology of rumen ciliate protozoa**, Florida: CRC Press Inc, 1993, 96 p.

DENNIS, S.M.; NAGARAJA, T.G.; BARTLEY, E.E. Effects of lasalocid or monensin on lactate-producing or -using bacteria, **Journal of Animal Science**, v.52, p.418-426, 1981.

DINI, Y.; GERE, J.; BRIANO, C.; MANETTI, M.; JULIARENA, P.; PICASSO, V. 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, 2012.

DOHME, F.; MACHMÜLLER, A.; WASSERFALLEN, A.; KREUZER, M. Ruminal methanogenesis as influenced by individual acids supplemented to complete ruminant diets. **Letters in Applied Microbiology**, v. 32, p.47–51, 2001.

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.

DUCKETT, S.K.; GILLIS, M.H. Effects of oil source and fish oil addition on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. **Journal of Animal Science**, v.88, p.2684–2691, 2010.

EIFERT, E.D.C.; LANA, R.D.P.; LANNA, D.P.D. Effects of dietary supplementation of monensin and soybean oil on production of early lactating dairy cows. Efeitos do fornecimento de monensina e óleo de soja na dieta sobre o desempenho de vacas leiteiras na fase inicial da lactação. **Revista Brasileira de Zootecnia**. v.34, p.2123-2132, 2005.

ELMEDDAH, Y.; DOREAU, M.; ROUEL, J.; CHILLIARD, Y. Effects of calcium salt supplementation on dairy cow performances in early lactation, Influence of the nature of concentrates. **Ann, Zootech**, v.43, p.341-353, 1994.

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.44, n.9, p.1768-1771, 1961.

FINLAY, B.J.; ESTEBAN, G.; CLARKE, K.J.; WILLIAMS, A.G.; EMBLEY, T.M.; HIRT, R.P. Some rumen ciliates have endosymbiotic methanogens, **FEMS Microbiology Letters**, v.117, p.157-162, 1994.

FIRKINS, J.L.; YU, Z.; MORRISON, M. Ruminant nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy. **Journal of Dairy Science**, v.90 (Suppl. 1), p1-16, 2007.

FOLCH, J.; LEES, M.; SLOANE-STANLEY, G.H. A simple method for the isolation and purification of total lipides from animal tissues. **The Journal of Biological Chemistry**, Baltimore, v.226, p.497-509, 1957.

FOLDAGER, J. **Protein requirement and non protein nitrogen for high producing cow in early lactation**, 1977, Tese (Doutorado em Ciência Animal) – Michigan State University, East Lasing, MI, 1977.

FRANZOLIN, R.; FRANZOLIN, M.H.T. População protozoários ciliados e degradabilidade ruminal em búfalos e bovinos zebuínos sob dieta à base de cana-de-açúcar. **Revista Brasileira de Zootecnia**, v.29, n.6, p.1853-1861, 2000.

GARRETT, W.N. Energy utilization by growing cattle as determined in 72 comparative slaughter experiments. **Energy metabolism**. Proc. Symp. v.26, p.3-7, 1980.

GEHMAN, A.M.; KONONOFF, P.J.; MULLINS, C.R.; JANICEK, B.N. Evaluation of nitrogen utilization and the effects of monensin in dairy cows fed brown midrib corn silage. **Journal of Dairy Science**, v. 91, p.288-300, 2008.

GERBER, P.; STEINFELD, H.; HENDERSON, B.; MOTTET, A.; OPIO, C.; DIJKMAN, J.; FALCUCCI, A.; TEMPIO, G. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. **Food and Agriculture Organization of the United Nations (FAO)** (Eds.). Rome, Italy. pp.116, 2013.

GOMES, C.T. **Aditivos (monensina sódica, levedura e probióticos) para bovinos da raça Nelore terminados com rações com concentrado rico em co-produtos**, 2009, 109 p, Dissertação (Mestrado em Ciência Animal e Pastagens) - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, 2009.

GREEN, B.L.; MCBRIDE, B.W.; SANDALS, D.; LESLIE, K.E.; BAGG, R.; DICK, P. The impact of a monensin controlled-release capsule on subclinical ketosis in the transition dairy cow. **Journal of Dairy Science**, v.82, p.333-342, 1999.

GUAN, H.; WITTENBERG, K.M.; OMINSKI, K.H. Efficacy of ionophores in cattle diets for mitigation of enteric methane. **Journal of Animal Science**, v.84, n.7, p.1896-1906, 2006

GUYADER, J.; EUGENE, M.; NOZIERE, P.; MORGAVI, D.P.; DOREAU, M.; MARTIN C. Influence of rumen protozoa on methane emission in ruminants: a meta-analysis approach. **Animal**, v. 8, p.1816–1825, 2014.

HAIMOUD, A.D.; VERNAY, M.; BAYOURTHE, C. et al. Avoparcin and monensin effects on the digestion of nutrients in dairy cows fed a mixed diet. **Canadian Journal of Animal Science**, v.75, n.3, p.379-385, 1995.

HARVATINE, K.J.; ALLEN, M.S. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows, **Journal of Dairy Science**, v.88, p.4018-4027, 2005.

HARVATINE, K.J.; ALLEN, M.S. Effects of fatty acid supplements on ruminal and total tract nutrient digestion in lactating dairy cows. **Journal of Dairy Science**, v.89, p.1092-1103, 2006.

HEGARTY, R.S. Reducing rumen methane emissions through elimination of rumen protozoa, **Australian Journal of Agricultural Research**, v.50, p.1321-1327, 1999.

HESS, B.W.; MOSS, G.E.; HULE, D.C. A decade of developments in the area of fat supplementation research with beef cattle and sheep. **Journal of Animal Science**, v.86, p.E188-E204, 2008.

HOLLMANN, M.; POWERS, W.J.; FOGIEL, A.C.; LIESMAN, J.S.; BELLO, N.M.; BEEDE, D.K. Enteric methane emissions and lactational performance of Holstein cows fed different concentrations of coconut oil. **Journal of Dairy Science**, v.95, p.2602-2615. 2012.

HRISTOV, A.N.; JOUANY, J.P. Factors affecting the efficiency of nitrogen utilization in the rumen. in: A.N. Hristov, E. Pfeffer (Eds.) **Nitrogen and Phosphorus Nutrition of Cattle and Environment**. CAB International, Wallingford, UK; 2005: 117–166.

HRISTOV, A.N.; IVAN, M.; RODE, L.M.; McALLISTER, T.A. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium or high-concentrate barley-based diets. **Journal of Animal Science**, v.79, n.2, p.515-524, 2001.

HRISTOV, A. N.; IVAN, M.; McALLISTER, T.A. In vitro effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high concentrate, barley based diet. **Journal of Animal Science**, v.82, p.2693–2704, 2004.

IKWUEGBU, O.A; SUTTON, J.D. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep, **British Journal of Nutrition**, v.48, p.365, 1982.

IQBAL, M.F.; CHENG, Y.F.; ZHU, W.Y.; ZESHAN, B. Mitigation of ruminant methane production: current strategies, constraints and future options. **World Journal of Microbiology and Biotechnology**, v.24, p.2747–2755, 2008.

IVAN, M.; MIR, P.S.; KOENIG, K.M. et al, Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep, **Small Ruminant Research**, v.41, p.215-227, 2001.

JENKINS, T.C.; McGUIRE, M.A. Major advances in nutrition: impact on milk composition. **Journal of Dairy Science**, v.89, p.1302-1310, 2006.

JENKINS, T.C.; PALMQUIST, D.L. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations, **Journal of Dairy Science**, v.67, p.978, 1984.

JENKINS, T.C.; WALLACE, R.J.; MOATE, P.J. et al. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. **Journal of Dairy Science**, v.86, p.397-412, 2008.

JENKINS, T.C., Fat interactions in ruminant diets, Page 117, in **Proc, 49th Minnesota Nutr, Conf.**, Univ, Minnesota, Bloomington, 1988.

JOHNSON, K.A.; JOHNSON, D.E. Methane Emissions from Cattle, **Journal Animal Science**, v.73, p.2483-2492, 1995.

JOHNSON, C.E. Soil nitrogen status 8 years after whole-tree clearcutting. **Canadian Journal of Forest Research**, v.25, p.1346-1355, 1995a.

JOHNSON, D.W. Soil properties beneath Ceanothus and pine stand in the eastern Sierra Nevada. **Soil Science Society of America Journal**, v.59, p.918-924, 1995b.

JORDAN, E.; LOVETT, D.K.; MONAHAN, F.H.; CALLAN, J.; FLYNN, B.; O'MARA, F.P. Effect of refined coconut oil or copra meal on methane output, and on intake and performance of beef heifers. **Journal Animal Science**, v.84, p.162-170, 2006.

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.

KIM, M.; Yu, Z. Variations in 16S rRNA-based microbiome profiling between pyrosequencing runs and between pyrosequencing facilities. **Journal of Microbiology**. (in press), 2014.

KNAPP, J.R.; LAUR, G.L.; VADAS, P.A.; WEISS, W.P.; TRICARICO, J.M. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions, **Journal of Dairy Science**, v.97, n.6, p.3231-3261, 2014.

KOENIG, K.M.; NEWBOLD, C.J.; MCINTOSH, F.M.; RODE, L.M. Effects of protozoa on bacterial nitrogen recycling in the rumen. **Journal Animal Science**, v.78, p.2431-2445, 2000.

KRAJCARSKI-HUNT, H.J.C.; PLAIZIER, J.P.; WALTON, R.; SPRATT, B.W.; MCBRIDE, B.W. Effect of subacute ruminal acidosis on in situ fiber digestion in lactating dairy cows. **Journal of Dairy Science**, v.85, p.570-573. 2002.

KRAMER, J.K.G.; FELLNER, V.; DUGAN, M.E.R. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. **Lipids**, Heidelberg, v.32, p.1219-1228, 1997.

KREUZER, M.; KIRCHGESSNER, M.; MFILLER, H.L. Effect of defaunation on the loss of energy in wethers fed different quantities of cellulose and normal or steamflaked maize starch, **Animal Feed Science and Technology**, v.16, p.233-241, 1986.

KULASEK, G.A. A micromethod for determination of urea in plasma, whole blood and blood cells using urease and phenol reagent, **Polskie Archiwum Weterynaryjne**, v.15, n.4, p. 801-810, 1972.

LASSEY, K.R. Livestock methane emission and its perspective in the global methane cycle. **Australian Journal of Experimental Agriculture**, v.48, n.2, p.114–118, 2008.

LOMAS C., 2013 – Farmers fight cow farts to protect the climate. **Erişim adresi:** <http://www.dw.de/farmers-fight-cow-farts-to-protect-the-climate/a-16702813>

MACHADO, F.S.; PEREIRA, L.G.R.; GUIMARÃES, R.JR.; LOPES, F.C.F.; CHAVES, A.V.; CAMPOS, M.M.; MORENZ, M.J.F. Emissões de metano na pecuária: conceitos, métodos de avaliação e estratégias de mitigação / Fernanda Samarini Machado. [et al.], – Juiz de Fora: **Embrapa Gado de Leite**. 2011, 92p. (Embrapa Gado de Leite, Documentos, 147).

MACHMULLER, A.; KREUZER, M. Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep, **Canadian Journal of Animal Science**, v. 79, p.65-72, 1999.

MACHMÜLLER, A.; OSSOWSKI, D.; WANNER, M.; KREUZER, M. Potential of various fatty feeds to reduce methane release from rumen fermentation in vitro (Rusitec) **Animal Feed Science and Technology**, v.71, p.117–130, 1998.

MARTIN, C.; MORGAVI, D.P.; DOREAU, M. Methane mitigation in ruminants: from microbe to the farm scale. **Animal**, v.4, p.351–365, 2010.

MARTIN, C.; MORGAVI, D.P.; DOREAU, M. Methane mitigation in ruminants: from microbes to the farm scale, **Animal**, v.4, p.351-365, 2009.

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: effect of monensin, sunflower oil, enzymes, yeast, and fumaric acid. **Journal Animal Science**, v.82, p.3346-3356, 2004.

MORGAVI, D.P.; FORANO, E.; MARTIN, C.; NEWBOLD, C.J. Microbial ecosystem and methanogenesis in ruminants. **Animal**, v.4, p.1024–1036, 2010.

MORRIS, F.E.; BRANINE, M.E.; GALYEAN, M.L; HUBBERT, M.E.; FREEMAN, A.S.; LOFGREEN, G.P. Effects of rotating monensin plus tylosin and lasalocid on

performance, ruminal fermentation, and site and extent of digestion in feedlot cattle. **Journal Animal Science**, v.68, p.3069–3078, 1990.

MOSS, A.R.; JOUANY, J.P.; NEWBOLD, J. Methane production by ruminants: its contribution to global warming. **Annales de Zootechnie**, v.49, n.3, p.231-254, 2000.

MOYA, D.; MAZZENGA, A.; HOLTSHAUSEN, L.; COZZI, G.; GONZÁLEZ, L.A.; CALSAMIGLIA, S.; GIBB, D.G.; MCALLISTER, T.A.; BEAUCHEMIN, K.A.; SCHWARTZKOPF-GENSWEIN, K. Feeding behavior and ruminal acidosis in beef cattle offered a total mixed ration or dietary components separately, **Journal Animal Science**, v.89, p.520-530, 2011.

MUNTIFERING, R.B.; THEURER, B.; NOON, T.H. Effects of monensin on site and extent of whole corn digestion and bacterial protein synthesis in beef steers. **Journal Animal Science**, v.53, p.1565-1573, 1981.

MUTSVANGWA, T.; WALTON, J.P.; PLAIZIER, J.C.; DUFFIELD, T.F.; BAGG, R.; DICK, P.; VESSIE, G; MCBRIDE, B.W. Effects of a monensin controlled-release capsule or premix on attenuation of subacute ruminal acidosis in dairy cows. **Journal of Dairy Science**, v.85, p.3454–3502, 2002.

MYERS, W.D.; LUDDEN, P.A.; NAYIGIHUGU, V.; HESS, B.W. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. **Journal Animal Science**, v.82, n.1, p.179-832, 2004.

NAGARAJA, T.G.; AVERY, T.B.; BARTLEY, E.E.; ROOF, S.K.; DAYTON, A.D. Effect of lasalocid, monensin, or thiopeptin on lactic acidosis in cattle, **Journal of Animal Science**, v.54, p.649-658, 1982.

NAGARAJA, T.G.; AVERY, T.B.; GALITZERT, S.J.; HOLMAN, D.L. Effect of ionophore antibiotics on experimentally induced lactic acidosis in cattle. **American Journal of Veterinary Research**, v.46, p.2444–2452, 1985.

NAGARAJA, 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, p,523-632, 1997.

NEWBOLD, C.J.; WALLACE, R.J.; WALKER, N.D. The effect of tetronasin and monensin on fermentation, microbial numbers and the development of ionophorer-resistant bacteria in the rumen. **Journal of Applied Microbiology**, v.75, p.129-134. 1993.

NEWBOLD, C.J.; LASSALAS, B.; JOUANY, J.P. The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro, **Letters in Applied Microbiology**, v.21, n.4, p.230-234, 1995.

NICHOLSON, T.; OMER, S.A. The inhibitory effect of intestinal infusions of unsaturated long-chain fatty acids on forestomach motility of sheep, **British Journal of Nutrition**, v.50, p.141–149, 1983.

NRC, NATIONAL RESEARCH COUNCIL. 2000. **Nutrient Requirements of Beef Cattle**: Seventh Revised Edition: Update 2000. Washington, DC: The National Academies Press. 248 p.

NRC. NATIONAL RESEARCH COUNCIL, **Nutrient requirements of dairy cattle**, 7th ed, Washington: National Academies Press, 2001, 381p.

ODONGO, N.E.; BAGG, R.; VESSIE, G.; DICK, P.; OR-RASHID, M.M.; HOOK, S.E.; GRAY, J.T.; KEBREAK, E.; FRANCE, J.; MCBRIDE, B.W. Long-term effects of feeding monensin on methane production in lactating dairy cows. **Journal of Dairy Science**, v.90, p.1781-1788, 2007.

ØRSKOV, E.R.; DEB HOVELL, F.D.; MOULD, F. The use of the nylon bag technique for evaluation of feedstuffs, **Tropical Animal Production**, v.5, n.3, p.195-213, 1980.

OSBORNE, J.K.; MUTSVANGWA, T.; ALZAHAL, O.; DUFFIELD, T.F.; BAGG, R.; DICK, P.; VESSIE, G.; MCBRIDE, B.W. Effects of monensin on ruminal forage degradability and total tract diet digestibility in lactating dairy cows during grain-induced subacute ruminal acidosis. **Journal of Dairy Science**, v.87, p.1840–1847, 2004.

OWENS, F.N.; BASALAN, M. Ruminal Fermentation. In: MILLEN D.D.; ARRIGONI, M.D.B.; PACHECO, R.D.L. **Rumenology**. Switzerland: Springer, 2016. p.63-102.

OWENS, F.N.; SHOCKEY, B.; FENT, R.W.; RUST, S.R. Monensin and abomasal protein passage of steers. **Journal of Animal Science**, v.47(Suppl. 1), p.114. (Abstr.), 1978.

PATRA, AK. Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. **Environmental Monitoring and Assessment**. v.184, p.1929 –1952, 2012.

PAUL, R.G.; WILLIAMS, A.G.; BUTLER, R.D. Hydrogenosomes in the rumen entodiniomorphid ciliate *Polyplastron multivesiculatum*. **Journal of General Microbiology**, v.136, p.388-396, 1990.

PENNER, G.B.; BEAUCHEMIN, K.A.; Variation among cows in their susceptibility to acidosis: challenge or opportunity? **Advanced Dairy Technologies**, v.22, p.173–187. 2010.

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.

PENNER, G.B.; BEAUCHEMIN, K.A.; MUTSVANGWA, T. Severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. **Journal of Dairy Science**, v.90, p.365–375, 2007.

PERNA JUNIOR, F.; CASSIANO, E.C.O.; MARTINS, M.F.; ROMERO, L.A.; ZAPATA, D.C.V.; PINEDO, L.A.; MARINO, C.T.; RODRIGUES, P.H.M. Effect of tannins-rich extract from *Acacia mearnsii* or monensin as feed additives on ruminal fermentation efficiency in cattle. **Livestock Science**, v.203, p.21-29, 2017.

PLAIZIER, J.C.; KEUNEN, J.E.; WALTON, J.P.; DUFFIELD, T.F.; MCBRIDE, B.W. Effect of subacute ruminal acidosis on in situ digestion of mixed hay in lactating dairy cows. **Canadian Journal of Animal Science**, v.81, p.421–423, 2001.

PLAIZIER, J.C.; MARTIN, A.; DUFFIELD, T.; BAGG, R.; DICK, P.; MCBRIDE, B.W. Effect of prepartum administration of monensin in a controlled-release capsule on apparent digestibilities and nitrogen utilization in transition dairy cows. **Journal of Dairy Science**, v.83, p.2918–2925, 2000.

RAVVA, S.V.; SARREAL, C.Z.; MANDRELL, R.E. Altered protozoan and bacterial communities and survival of *Escherichia coli* O157:H7 in monensin-treated wastewater from a dairy lagoon. **PloS one**, v.8(1), e54782, 2013.

RODRIGUES, P.H.M., PINEDO, L.A., ROMERO, L.A., PERNA JUNIOR, F., MARTINS, M.F., CASTRO, A. L., GODOY, 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: 49TH REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, Brasília, DF, Brazil, 2012.

ROGERS, M.; JOUANY, J.P.; THIVEND, P.; FONTENOT, J.P. The effects of short-term and long-term monensin supplementation, and its subsequent withdrawal on digestion in sheep. **Animal Feed Science and Technology**, v.65, p.113-127, 1997.

ROGERS, M.; JOUNANY, J.P.; THIVEND, P.; FONTENOT, J.P. Comparative effects of feeding and duodenal infusion of monensin on digestion in sheep. **Canadian Journal of Animal Science**, v.71, p.1125–1133, 1991.

RUSSELL, J.B.; HOULIHAN, A.J. Ionophore resistance of ruminal bacteria and its potential impact on human health. **FEMS Microbiology Reviews**, v.27, p.65– 74, 2003.

RUSSEL, J.B.; STROBEL, H.J. Effect of ionophores on ruminal fermentation, **Applied and Environmental Microbiology**, Washington, v.55, n.1, p.1- 6, 1989.

SAS, SAS/STAT-User's guide, 9,2, Cary, NC, USA: SAS Inst, Inc, 2010,

SEJIAN, V.; LAL, R.; LAKRITZ, J.; EZEJI, T. Measurement and prediction of enteric methane emission. **International Journal of Biometeorology**, 55: 1-16, 2011.

SILVA, M.M.C.; RODRIGUES, M.T.; RODRIGUES, C.A.F.; BRANCO, R.H.; LEÃO, M.I.; MAGALHÃES, A.C.M.; MATOS, R.S. Efeito da suplementação de lipídios sobre a digestibilidade e os parâmetros da fermentação ruminal em cabras leiteiras, **Revista Brasileira de Zootecnia**, v.36, p.246-256, 2007.

SIMPSON, M.E. Effects of certain antibiotics in vitro cellulose digestibility and volatile fatty acid production by ruminal microorganisms. **Journal of Animal Science**, v.47 (Suppl .I), p.429 (Abstr.), 1980.

TITGEMEYER, E.C.; ARMENDARIZ, C.K.; BINDEL, D.J.; GREENWOOD, R.H.; LOEST, C.A. Evaluation of titanium dioxide as a digestibility marker in cattle, **Journal of Animal Science**, v.79, p.1059–1063, 2001.

TOMKINS, N.W.; DENMAN, S.E.; PILAJUN, R.; WANAPAT, M.; MCSWEENEY, C.S.; ELLIOTT, R. Manipulating rumen fermentation and methanogenesis using an essential oil and monensin in beef cattle fed a tropical grass hay. **Animal Feed Science and Technology**, v.200, p.25-34, 2015.

TOWNE, G.; NAGARAJA, T.G.; BRANDT JR. R.T. et al, Ruminal ciliated protozoa in cattle fed finishing diets with or without supplemental fat, **Journal of Animal Science**, v.68, p.2150-2155, 1990.

UEDA, K.; FERLAY, A.; CHABROT, J.; LOOR, J.J.; CHILLIARD, Y.; DOREAU, M. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage:concentrate ratios, **Journal of Dairy Science**, v.86, p.3999–4007, 2003.

USEPA. 2007. US Environmental protection agency. Climate change and greenhouse gas emission. Available on line at: <http://epa.gov/climatechange/emissions/index.html>

USHIDA, K.; JOUANY, J.P.; DEMEYER, D.I. Effects of presence or absence of rumen protozoa on the efficiency of utilization of concentrate and fibrous feeds. In: **Physiological aspects of Digestion and Metabolism in Ruminants** (Tsuda T, Sasaki Y, Kawashima R, eds) Academic Press, Tokyo, p. 625-654, 1991.

VAN NEVEL, C.J.; DEMEYER, D.I. Control of rumen methanogenesis, **Environmental Monitoring and Assessment**, v.42, p.73-97, 1996.

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. 1991, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, n.10, p.3583–3597.

VASCONCELOS, A.M.; LEÃO, M.I.; VALADARES FILHO, S.C.; VALADARES, R.F.D.; DIAS, M.; MORAIS, D.A.E.F. Parâmetros ruminais, balanço de compostos nitrogenados e produção microbiana de vacas leiteiras alimentadas com soja e seus subprodutos, **Revista Brasileira de Zootecnia**, v.39, n.2, p.425–433, 2010.

VENDRAMINI, J.M.B.; SANCHEZ, J.M.D.; COOKE, R.F. et al. Stocking rate and monensin supplemental level effects on growth performance of beef cattle consuming warm-season grasses. **Journal of Animal Science**, v.93, p.3682-3689, 2015.

VERBIC, J.; CHEN, X.B.; MACLEOD, N.A. Excretion of purine derivatives by ruminants, Effect of microbial nucleic acid infusion on purine derivative excretion by steers, **Journal of Agricultural Science**, v.114, n.3, p.243-248, 1990.

WANG, L.A.; GOONEWARDENE, Z. The use of MIXED models in the analysis of animal experiments with repeated measures data, **Canadian Journal of Animal Science**, v.84, p.1-11, 2004.

WANG, Z. B.; XIN, H. S.; BAO, J.; DUAN, C.Y.; CHEN, Y.; QU, Y. L. Effects of hainanmycin or monensin supplementation on ruminal protein metabolism and populations of proteolytic bacteria in Holstein heifers. **Animal Feed Science and Technology**, v.201, p.99–103, 2015.

WEISS, W.P.; PINOS-RODRIGUEZ, J. M. Production responses of dairy cows when fed supplemental fat in low- and highforage diets. **Journal of Dairy Science**, v.92, p.6144-6155, 2009.

WILLIAMS, A.G.; COLEMAN, G.S. The rumen protozoa. P.N. Hobson, C.S. Stewart (Eds.), *The Rumen Microbial Ecosystem*, Blackie Academic and Professional, London, UK (1997), pp. 73-139.

ZINN, R.A.; GULATI, S.K.; PLASCENCIA, A.; SALINAS, J. Influence of ruminal biohydrogenation on the feeding value of fat in finishing diets for feedlot cattle. **Journal of Animal Science**, v.78, p.1738-1746, 2000.

CHAPTER IV.
KINETICS OF RUMEN DEGRADABILITY AND PASSAGE RATE OF
DRY MATTER AND NEUTRAL DETERGENT FIBER IN NELLORE
COWS FED DIETS SUPPLEMENTED WITH VEGETABLE OILS
AND MONENSIN

4. KINETICS OF RUMEN DEGRADABILITY AND PASSAGE RATE OF DRY MATTER AND NEUTRAL DETERGENT FIBER IN NELLORE COWS FED DIETS SUPPLEMENTED WITH VEGETABLE OILS AND MONENSIN

Abstract: The objective of the present experiment was to evaluate the effect of diet supplementation with vegetable oils and monensin for cattle on ingestive behavior variables (ingestion rate, rumination rate and chewing rate) and ruminal kinetics (passage rate, disappearance rate and degradability of dry matter, neutral detergent fiber and protein). Eight Nellore cows (494 ± 105 kg of BW) not pregnant and non-lactating ruminally cannulated (10 cm of diameter and 7.5 cm of thickness) non-pregnant and non-lactating were used in a 4 x 4 replicate Latin square experimental design with a 2 x 4 factorial arrangement of treatments (which refers to monensin and different sources of lipids inclusion) and 29 days per experimental period. The animals were randomly assigned to one of the eight treatments: Control (CONT), diet without lipid and monensin inclusion, canola (CAN), soybean (SOY) and sunflower (SUN). The inclusion of lipid sources in the diet was 3.5% of DM as oil. Besides vegetable oils inclusion, each cow in a square daily received 300 mg (approximately 32 mg / kg DM) of sodium monensin (Rumensin® 200, Elanco Saúde Animal, Brazil) during the whole experimental period, delivered twice a day (150 mg at 08h00 and 150 mg at 16h00), mixed with the diet. Diet adaptation (d 1-18) was followed by 11 days (d 19-29) of feed and water intake measurement. Ingestive behavior was evaluated during 24 h (d 19). *In situ* ruminal degradability (kd) was measured between days 20 to 24. Passage rate (kp) of DM was evaluated over a period of 48 hours (d 26 to 28). In order to evaluate ruminal kinetics and disappearance rate (kt), the rumen of each animal was emptied on consecutive days (d 28 and 29). Fiber ruminal kinetics were determined by indigestible NDF marker. The CAN treatment reduced the rumination rate of NDF, kg/min (22.2%). The potential degradability (PD, %) of DM and NDF were reduced, whereas the non degradable fraction (Ind, %) of DM and NDF were increased by the addition of lipids in the diet. This was related to the kt (%/h) of DM and NDF which decreased with the addition of lipid sources. Real degradability (RD, %) of DM was increased by SUN treatment at 15.4% compared to CONT, and, when monensin was added in the diet an increase of 13.1% RD of DM was observed compared to no addition of monensin. This was related to kp of DM (%/h) which decreased by 40.9% when monensin was added to the diet. Daily fecal flow was reduced by 13.9% by CAN when compared to the control treatment. A source rich omega-6 polyunsaturated fatty acids, such as sunflower oil, results in an increase in real dry matter

degradability, without impairing dry matter intake or feed passage rate. However, the combination of monensin and lipid source inclusion in diets did not result in either synergistic or antagonistic effect. Source of omega-3 polyunsaturated fatty acids (PUFAs), as soybean oil results in higher RD of CP and NDF. However, sources of monounsaturated fatty acids present in canola oil or sources of omega-6 PUFAs present in sunflower oil, reduce the effective and real degradability of NDF. The addition of monensin in cattle diets at a dose of 32 mg / kg of DM increases the DR of DM by 13%, however, it reduces the passage rate of this fraction. The present study indicates that the combination of monensin with sources of vegetable oils does not result in either synergistic, additive or antagonistic effect.

Key words: Disappearance rate. Passage rate. Ruminal degradability. Ruminal kinetics.

4.1 INTRODUCTION

The kinetics of dry matter (DM) degradation and its fractions (CP and NDF) and the behavior of passage rate together with ruminal retention time determine the net energy value of a diet. Dietary digestion in the rumen is the result of the competition between the rates of degradation and passage along the gastrointestinal tract. Different types of feed influence the rate of particle degradation and the distribution between small and large particles in the ruminal environment. The determination of real degradability (RD) of DM, CP and NDF is one of the most important tools in the ruminal kinetics study, since it allows to establish the true quantity of each of the fractions that are degraded in the rumen, taking in account the real passage rate determined by external marker.

Digestion and passage rates act simultaneously and competitively on rumen digesta removal, and it is necessary to study the combined effects of them in order to maximize the consumption of digestible nutrients. In this way, understanding how rumen volume, digestion rate and digestion rate respond to changes in the level of feed intake is essential to understand the mechanism by which rumen degradability and nutrient digestibility occurs (KOVÁCS et al., 1998).

Lipid supplementation in ruminant diets has a direct effect on DMI, increasing the retention time of the ingested fibrous particles in the rumen, due to a reduced passage rate through the gastrointestinal tract and a direct decrease in rumen fiber degradability, since lipids, especially unsaturated fatty acids, have a toxic effect on fibrolytic bacteria

(RICHARDSON et al., 1984; CHALUPA et al., 1986) and create a physical barrier on the surface of the fibrous particles, which prevents the action of microbial enzymes in NDF potentially digestible sites. Therefore, when lipids are added to ruminant diets, they can significantly affect ruminal fermentation, reducing diet degradability and digestibility, mainly of the fibrous fraction. Accordingly, with lipid inclusion, the digestion of structural carbohydrates in the rumen can be reduced by up to 50% or more (IKWUEGBU; SUTTON, 1982; JENKINS; PALMQUIST, 1984). However, the magnitude of dietary components degradation reduction in the rumen varies with the fatty acid profile of the lipid source and the quantity added in the diet (FERLAY; DOREAU, 1992). For monensin, its mechanism of action is well established effects such as: increase production of short chain fatty acids; modification of dry matter digestibility; influence on rumen filling and passage rate and; increase in animal production and performance (SCHELLING, 1984). Lemenager et al. (1978), Pond & Ellis (1981), Ellis & Delaney (1982) indicated that monensin decreases dry matter rate of passage in the rumen and increases the rate of digestion. However, the implications of these effects have not yet been clarified. This demonstrates that there is still insufficient data to firmly establish this relationship and, on the other hand, the effect of monensin on ruminal kinetic parameters must still be investigated.

In studies conducted with lipid sources on ruminal kinetics in cows, Valinote et al. (2006) identified that the potential degradation of sugarcane NDF was higher when cattle were supplemented with cottonseed, in relation to calcium salts of fatty acids. These authors also observed a 51.27% increase in effective degradability (ED%) with a 2% passage rate for cotton seed treatment. On the other hand, Zeoula et al. (2014) evaluated the supplementation of sodium monensin or probiotic, in a diet based on Tifton 85 hay, with a forage:concentrate ratio of 80:20 and observed that the presence of ionophore increased the potentially degradable fraction (b) of DM and CP in relation to the control diet. The authors indicated that the addition of monensin results in increased potential (PD) and ED degradability of CP for the different solids passage rates with addition of ionophore in relation to the control diet. However, the ED of DM for the 5% and 8%/h solids passage rates was reduced with the addition of the ionophore when compared to control diet.

From the previous paragraph, when it is considered that the addition of oil in cattle diet can result in an increase in feed retention time and in a reduction in ruminal degradability, mainly of fiber and, consequently, reduction of diet use, it becomes of

interest to study the combined action of monensin and vegetable oils in order to verify if there is any synergistic or at least additive effect that inhibits the negative effect of lipids (physical barrier on the surface of the fibrous particles) in the degradation and feed utilization.

From the point of view of carbohydrates fermentation in the rumen, the combination of monensin with vegetable oils is expected to improve dry matter ruminal degradation and its fractions (CP and NDF), due to the reduction of ruminal biohydrogenation process of lipids (VAN NEVEL; DEMEYER, 1995). This is a favorable effect because monensin reduces the potential negative effects of unsaturated fatty acids on ruminal fiber fermentation. It is also expected that the combination of these sources will result in a consequent reduction in the daily fecal flux by cattle.

The objective of this study was to evaluate the effect of cattle diet supplementation with vegetable oils and monensin on ingestive behavior variables (ingestion rate, rumination rate and chewing rate) and ruminal kinetics (passage rate, disappearance rate and dry matter, neutral detergent fiber or crude protein degradability) in Nellore cows, which allows to generate more accurate data on the extent of rumen digestion and the amount of potentially degradable material in feces.

4.2 MATERIALS AND METHODS

The present study was conducted in the Laboratory of Ruminant Nutrition of the College of Veterinary Medicine and Animal Science, University of Sao Paulo (USP), Pirassununga, Brazil. The experiment was conducted according the guidelines established by the Ethics Committee in the Use of Animals of the College of Veterinary Medicine and Animal Science, under the protocol N° 9236160216, in respect of ethical principles of animal experimentation for care and use of animals.

4.2.1 Animals, Treatments and Experimental design

Eight Nellore cows (494 ± 105 kg of body live weight) not pregnant and non-lactating, ruminally cannulated (10 cm of diameter and 7.5 cm of thickness) were randomly distributed to one of eight experimental diets, isoenergetic (1.2 Mcal of NE_g/kg of DM) and isoproteic (14% of CP), that differ by the lipid source used and the addition or not of sodium monensin (Rumensin® 200, Elanco Animal Health, Brasil). The treatments

were: Control (CONT), diet without lipid and monensin inclusion, canola (CAN), soybean (SOY) and sunflower (SUN). The inclusion of lipid sources in the diet was 3.5% of DM as oil. Besides vegetable oils inclusion, each cow in a square daily received 300 mg (approximately 32 mg / kg DM) of sodium monensin (Rumensin® 200, Elanco Saúde Animal, Brazil) during the whole experimental period, delivered twice a day (150 mg at 08h00 and 150 mg at 16h00), mixed with the diet.

The experimental design was a replicated 4x4 Latin square, in a factorial arrangement of treatments 2 x 4 (regarding inclusion or not of sodium monensin and lipids source inclusion in diets). The experimental unit was the animal inside each period, totalizing 32 experimental units. Each experimental period had 29 days (18 days for diet adaptation and 11 days for data collection) totalizing 116 days.

Diets were offered twice a day, at 08h00 and 16h00 as a total mixed ration (60:40, forage:concentrate ratio), for *ad libitum* intake (5 - 10% of leftovers). In all diets the forage source used was corn silage. Forage and concentrate feeds were weekly sampled for DM content determination and diets were adjusted considering their variation in DM content. The amount of forage and concentrate daily offered was adjusted depending on leftovers from the day before. Diets were formulated to attend nutritional requirements for beef cattle, according NRC (2000). The proportion of ingredients and chemical composition are described at Table 15.

For determination of fatty acids profile of oils included in experimental diets (Table 16), the extraction was performed by methodology described by Folch et al. (1957), by sample homogenization with a solution composed by chloroform and metanol 2:1 in homogenizer Ultra Turrax Marconi®. Lipids were isolated after the addition of NaCl 1.5% solution. For methylation, methyl esters were formed according methodology described by Kramer et al. (1997). Fatty acids were quantified by gas chromatography (CG-2010 Plus - Shimadzu, auto injetor AOC 20i), using cappillary column SP-2560 (100 m × 0.25 mm of diameter with 0.02 mm of thickness, Supelco, Bellefonte, PA). The initial temperature of column was 45°C, with progressive heating until reaching 175°C, remaining for 27 minutes. After, a further increase of 4 °C/min was started up to 215°C, remaining for 35 minutes. Hydrogen (H₂) was used as a carrier gas with flux of 40 cm³/s. The temperature used by the flame ionization detector (FID) was 250°C, H₂ flow of 40 mL/min, air flow of 400 mL/min, make-up of 30 mL/min kPa (N₂) and sampling rate of 40 msec. The total time of each run per sample (stop time) was 86 minutes.

Table 15 - Proportion of ingredients and chemical composition of experimental diets expressed as percentage of dry matter (% DM).

Item	Experimental diets ¹			
	CONT	CAN	SOY	SUN
Ingredients, % DM				
Corn silage	60.9	61.9	61.9	61.9
Ground corn grain	22.6	14.0	14.0	14.0
Soybean meal	13.4	17.5	17.5	17.5
Vegetal oil	-	3.5	3.5	3.5
White salt	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.1	0.1	0.1	0.1
Limestone	0.5	0.5	0.5	0.5
Mineral mixture ²	2.0	2.0	2.0	2.0
Chemical composition				
Dry matter, %	54.1	53.8	53.8	53.8
CP, % of DM	14.6	14.9	14.6	14.1
RDP, % of CP ³	66.8	69.4	69.4	69.4
RUP, % of CP ³	33.2	30.6	30.6	30.6
NDF, % of DM	40.6	38.1	41.8	40.3
ADF, % of DM	26.0	24.7	29.4	29.6
NFE, % of DM	34.6	33.3	31.7	30.0
Ash, % of DM	7.1	7.3	7.3	7.3
Ca, % of DM	0.89	1.07	1.07	1.07
P, % of DM	0.30	0.29	0.29	0.29
EE, % of DM	3.1	6.4	6.3	6.6
TDN, % of DM	76.3	78.1	78.0	77.6
NE _m , Mcal/kg of DM ⁴	1.83	1.88	1.88	1.87
NE _g , Mcal/kg of DM ⁴	1.20	1.25	1.24	1.23

¹CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil. Monensin was included in respective diets at 300 mg per animal per day – approximately 32 mg/kg of DM; ²Mineral and vitamin premix, composition per kg of product: 200 g of Ca, 60 g of P, 20 g of S, 20 g of Mg, 70 g of Na, 15 mg of Co, 700 mg of Cu, 700 mg of Fe, 40 mg of I, 1,600 mg of Mn, 19 mg of Se, 2,500 mg of Zn; ³Values estimated by the software *Nutrient Requirements of Beef Cattle: Seventh Revised Edition (2000)*. ⁴ME (Metabolizable energy) = [(TDN kg x 0.04409) x 0.82] x 100 (NRC, 2000); equations to convert ME values into NE_m and NE_g (Mcal/kg DM) have been reported by Garrett (1980) and NRC (2000).

Fatty acids were identified according retention time of methyl esters of samples using the standard solutions 463 Nu-Chek®, vaccenic acid C18:1 trans-11 (V038-1G, Sigma®), C18:2 trans-10 cis-12 (UC-61M 100mg), CLA and C18:2 cis-9, trans-11 (UC-60M 100mg) and tricosanoic acid (Sigma®). Fatty acids were quantified by normalization

of the peak area of the methyl esters, using the GS solution software 2.42®. Fatty acids were expressed as percentage of total methyl ester quantified.

Table 16. Fatty acids profile of oils used in experimental diets.

Fatty acid ¹	Canola oil	Sunflower oil	Soybean oil
Lauric, C12:0	0.021	0.008	0.001
Myristic, C14:0	0.061	0.065	0.079
Palmitic, C16:0	4.445	6.148	11.09
Palmitoleic, C16:1 trans 9	0.036	0.013	0.008
Palmitoleic, C16:1 cis 9	0.219	0.091	0.080
Heptadecanoic, C17:0	0.080	0.038	0.071
Heptadecenoic, C17:1 cis 10	0.115	0.027	0.042
Stearic, C18:0	2.334	3.870	3.330
Oleic, C18:1 n-9 cis	56.23	37.67	21.45
Vaccenic, C18:1 cis 11	6.294	0.132	2.389
Linoleic, C18:2 n-6 cis	18.69	50.00	53.38
γ-linolenic, C18:3 n-6	0.421	0.010	0.097
Linolenic, C18:3 n-3	8.546	0.242	6.427
Araquidic, C20:0	0.020	-	0.317
Eicosenoic, C20:1 cis 5	0.062	0.007	0.022
Eicosenoic, C20:1 cis 8	0.365	0.004	0.083
Eicosadienoic, C20:2 n-6 cis 11,14	0.045	0.005	0.032
Eicosatrienoic, C20:3 n-6 cis 8, 11, 14	0.363	0.662	0.421
Eicosatrienoic, C20:3 n-3 cis 11, 14, 17	0.043	0.014	0.005
Heneicosanoic, C21:0	0.011	-	0.020
Tricosanoic, C23:0	0.021	0.019	0.038
Eicosapentaenoic, C20:5 n-3 cis (EPA)	0.162	0.188	0.125
Docosatrienoic, C22:3 n-3 cis 13, 16, 19	0.123	0.015	0.015
Docosatetraenoic, C22:4 n-6	0.009	-	-
Docosahexaenoic, C22:6 n-3 cis (DHA)	0.008	-	0.005
PUFAS ²	28.41	51.14	60.51
MUFAS ³	63.32	37.94	24.09
SFAS ⁴	7.011	10.16	14.95
n-3	8.882	0.459	6.577
n-6	19.52	50.67	53.93
n-6/n-3	2.197	110.3	8.199

¹Fatty acids expressed in percentage (%) of the sample; ²PUFA: Polyunsaturated fatty acids; ³MUFA: Monounsaturated fatty acids; ⁴SFA: Saturated fatty acids.

4.2.2 Dry matter and water intake

Dry matter intake (DMI) was daily measured during the last eleven days of each experimental period. Leftovers were collected and weighed every morning at 06h30, in order to adjust the quantity of forage and concentrate daily offered. When there was no leftover, offered diet was raised in 10%. If there was a surplus between 5 - 10%, diet was kept at the same level and if the surplus was higher than 10%, offered diet was reduced in 10%. Dry matter intake was estimated by the difference between the quantity of diet supplied in one day and the leftover, collected and weighed in the morning following the offering, multiplied by the percentage of dry matter of the diet.

Simultaneously, water intake was measured by a hydrometer (L/d). Water intake in relation to dry matter intake (L/kg DMI), water intake in relation to body live weight (% of BLW) and metabolic weight (mL/kg of MW) were calculated from the primary value.

Samples of feed used in diets and of collected feces (approximately 200 g, per treatment and per period) were dried at 55°C for 72 h in a forced air circulation oven and ground (1.0 mm particle size in Willye type mill) for determination of DM (Method 930.15), mineral matter (MM - Method 942.05), crude protein (CP - Method 984.13) by total N determination ($N \times 6.25$) using the micro-Kjeldahl technique, calcium (Ca - Method 968.08) and phosphorus (P - Method 965.17), according AOAC (2000), ether extract (EE - Method Am 5-04) by ANKOM XT15 Extractor® equipment, according AOCS (2005), neutral detergent fiber (NDF) and acid detergent (ADF), according Van Soest et al. (1991). These samples were performed with the objective to evaluate nutrient composition of offered diet, as well as, intake and digestibility of each nutrient in diet.

4.2.3 Ingestive behavior

Ingestive behavior was evaluated on day 19, during a 24-hour uninterrupted time, beginning at 08h00, through visual monitoring. The following parameters were observed at each 5 minutes: eating (E), drinking (D), ruminating (R) and idleness (I), according to the methodology cited by Maekawa et al. (2002). In relation to the chewing (C), this was calculated by the sum of ruminating and eating parameters. During the nocturnal observation of the animals, the environment was maintained with a soft artificial lighting.

Results regarding ingestive behavior factors were obtained using equations, where the sum of all feeding events represented the daily number of events (NE) eating (NEE,

events/d). The other activities, such as the number of events drinking (NED), ruminating (NER) and in idleness (NEI) were calculated in the same way. Regarding the daily number of chewing events (NEC, events/d), this was obtained by the sum of NEE and NER. The event was considered the sum of three or more activities followed during the visual evaluation performed every 5 minutes.

Total time in activity (TTA) was defined as the sum of the activities (number of activities/d) of each of the 5-minute duration parameters in which each animal spent feeding or performing other evaluated activities (drinking, ruminating or idleness), multiplied by the constant 5, referring to the interval of time of evaluation of the ingestive behavior. Total time chewing (TTC, min/d) was calculated by the sum of the total time eating (TTE) and total time ruminating (TTR). Regarding the mean time per event (MTE, min/event) for each of the analyzed activities, this was obtained by its respective TTA divided by the activity NE. The mean time of chewing per event (MTC, min/event) was calculated by the ratio between TTC and NEC.

Dry matter and neutral detergent fiber intake rate (kg/min) were calculated by the intake of each of these fractions on the day of the evaluation divided by the TTE. When this was evaluated in minute/kg, TTE was divided by DM or NDF intake. Ingestion rate (DM or NDF) per event (kg/event) was calculated as the ratio between the intake of these fractions on the day of evaluation and the NEE. Regarding rumination (TTR) and chewing rates (TTC) of DM and NDF (kg/min or min/kg), these variables were calculated in a similar way to the ingestion rate in which the ingested amounts of these fractions were divided by TTR or TTC, or the division was performed inversely, respectively. For calculation of the TTR or the TTC per event, it was done in a similar way as indicated above for the determination of the intake rate per event, but considering the NER and NEC, respectively.

4.2.4 Percentage of particle size distribution and diet selection index

From day 18 to day 24, samples were collected from the total diet (immediately after delivery) and the leftovers from the following day of each animal to determine the percentage of particle distribution and to analyze the extent of diet selection by determining the preference index using the Penn State Particle Separator (PSPS) method as described by Heinrichs & Kononoff (1996). Penn State Particle Separator was equipped with two boxes containing at the bottom of each one, sieves with different diameters (19.0

and 8.0 mm), arranged one under the other from the largest to the smallest diameter. Besides, it has another box with solid bottom totaling three boxes.

For this analysis, approximately 500 g of each sample of the diet offered and the leftovers from each animal at each period were placed on the first box (19 mm diameter), which was shaken as described by Heinrichs & Kononoff (1996). After that, the fresh fractions of the samples retained in each sieve and in the box were weighed, and thus, the percentage distribution of the particles of the offered diet and the leftovers according to size was determined. Subsequently, from the data obtained, diet selection index of was calculated for each portion retained in each sieve, individually, by the following equation:

$$\text{Selectivity index} = \frac{\text{Actual ingestion}}{\text{Expected ingestion}} \quad (1)$$

The actual intake and the expected intake were obtained using the following equations:

$$\text{Actual ingestion} = \text{QDO} \times \text{DPS}_{\text{TD}} - (\text{QL} \times \text{DPS}_{\text{L}}) \quad (2)$$

where:

QDO = Quantity of diet offered (kg);

DPS_{TD} = Distribution of particle size of total diet (%);

QL = Quantity of leftovers (kg);

DPS_L = Distribution of particle size of leftovers (%).

$$\text{Expected ingestion} = \text{DPS}_{\text{TD}} \times \text{AIFM} \quad (3)$$

where:

DPS_{TD} = Distribution of particle size of total diet (% of fresh matter);

AIFM = Actual ingestion of fresh matter (kg).

Selection index smaller, greater than or equal to 1 indicate selection against, favor or absence of selection, respectively. For each box, an index was calculated independently of the other boxes (LEONARDI; ARMENTANO, 2003).

4.2.5 Ruminal kinetics variables

4.2.5.1 *In situ* ruminal degradability

Ruminal degradability was evaluated using the nylon bag technique, proposed by Ørskov et al. (1980), from days 20 to 24, in order to determine DM and its fractions (CP and NDF) disappearance of diets (forage:concentrate ratio = 60:40).

Samples of the ingredients of each of the diets were pre-dried in a forced air circulation oven at 55°C for 72 hours, milled in sieves with 2 mm, weighed and conditioned in nylon bags (10 x 20 cm) with a porosity of 50 µm (10 g DM, maintaining 60:40 ratio of total diet offered to animals). The bags were identified by animal, treatment and incubation time. After the preparation of the nylon bags, they were introduced through ruminal cannula and incubated in the ventral part of the rumen of each animal for 0, 3, 6, 12, 24, 48, 72 and 96 hours. After each incubation period, the bags were removed and washed in running water to ensure removal of the soluble material and that the pores were cleaned. After that, the bags were subjected to forced air ventilation drying at 55°C for 72 hours and weighed again, to determine the rate of disappearance of DM in the rumen. The remaining residues in the bags were analyzed for CP (AOAC, 2000) and NDF (VAN SOEST et al., 1991), in order to determine the rate of disappearance of these fractions.

In situ degradability data of DM, CP and NDF were obtained by the difference between the initial and final weight at ruminal incubation and expressed as percentage. Rumen degradation curves of DM, CP and NDF were adjusted to a non-linear regression using the NLIN procedure of the SAS (version 9.4), according to the equation proposed by Ørskov & McDonald (1979):

$$P = a + b (1 - e^{-ct}) \quad (4)$$

where:

P = quantity of nutritive component degraded at time “t”;

a = intercept of the degradation curve when t = 0, which corresponds to the water-soluble and completely degradable fraction of the analyzed nutrient component, which leaves rapidly from the nylon bag;

b = fraction of the analyzed nutrient component potentially degradable;

c = rate of degradation by fermentative action of b;

t = incubation time.

Potential Degradability (PD %) was given by $a + b$, which represented the amount of nutrient component analyzed (DM, CP and NDF) that can be dissolved and degraded in the rumen when time is not a limiting factor. In relation to the non-degradable fraction (Ind %), it was obtained from the PD (%) of the sample, given by $a + b$ (without being able to exceed the value of 100). From the above, the non-degradable fraction in the rumen was calculated by equation (5): $\text{Ind (\%)} = 100 - (a + b)$, as indicated by Ørskov et al. (1980).

After calculations of the coefficients a , b and c , these were applied in the equation proposed by Ørskov & McDonald (1979), to calculate the effective degradability (ED) of DM, CP and NDF at 2, 5, and 8 %/h, where:

$$\text{ED} = a + (b \times c) / c + k \quad (6)$$

ED = Effective ruminal degradability (2, 5 and 8 %/h) of the nutritive component analyzed;

k = Feed passage rate in the rumen. For these variables, values of particle passage rates used were recommended by Agricultural and Food Research Council (AFRC, 1993): 2 %/h = value generally used for animals with intake lower than maintenance; 5 %/h = value used for calves, dry cows and dairy cows with production lower than 15 kg of milk/d; and 8 %/h = value used for dairy cows producing more than 15 kg of milk. However, in the present experiment dry cows were used, but the three mentioned constants were used.

For real degradability (RD), the same equation for ED determination (ØRSKOV; McDONALD, 1979) was used. However, the actual passage rate value, evaluated by external marker, was used:

$$\text{RD} = a + (b \times c) / c + k \quad (7)$$

where:

RD = Real ruminal degradability of nutritive component analyzed;

k = Feed passage rate in rumen determined by external marker.

Real degradability represented the amount of the nutrient component analyzed (DM, CP, or NDF) that was degraded in the rumen and was defined by the time during which the nutrient was present in the rumen.

4.2.5.2 Dry matter ruminal passage rate

Dry matter passage rate of the experimental diets was evaluated during days 26 to 28 of each experimental period. In each period, 20 g of chromium oxide were infused in a single dose, via the ruminal cannula. The ruminal contents were collected before (0), 2, 4, 6, 9, 12, 24, 36 and 48 hours after infusion. Samples were immediately dried at 55 °C for 72 hours in a forced air ventilation oven and subsequently ground (1.0 mm particle size in a Willye type mill) and stored in glass vials for further analysis of DM and chromium oxide. Passage rate was calculated using the model proposed by Czerkawski (1986) by NLIN procedure of SAS (version 9.4):

$$Y = a.e^{-kt} \quad (8)$$

where:

Y = Marker concentration in time (t);

k = Passage rate in rumen (h⁻¹);

t = Time of sampling of the marker (h);

a = Marker concentration in initial time (t₀), assuming instant mixing to ruminal content (ppm);

e = Base of the neperian logarithm.

4.2.5.3 Ruminal dynamics and dry matter disappearance rate

On the last two days of each experimental period, the rumen of each animal was manually emptied (CHILIBROSTE et al., 2000), before (0 hours, time of lower rumen filling) and 3 hours (time of higher rumen filling) after morning feeding (ruminal mass and disappearance rate) of each animal submitted to the different diets.

Total ruminal content was removed via ruminal cannula and placed in plastic buckets (40 liters capacity). The separation of the solid content from the liquid was done by filtering the liquid portion through a sieve (2 mm) and subsequently the separated material in each bucket was weighed. Aliquots of 1 kg of solid content and liquid contents were removed and placed in previously identified aluminium trays. Immediately afterwards, the digesta was reconstituted and returned to the rumen. The samples (solid and

liquid contents) were dried at 55°C (forced air ventilation oven) for 72 hours in order to measure the DM content of each of the contents.

Total solid and liquid mass were corrected for their respective DM content and the sum of these components was used to determine the total ruminal mass. Ruminal disappearance rate of DM (Kt) expressed as percentage per hour (%/h) and kg per hour (kg/h) was calculated from dry matter ruminal content and DMI by the equations suggested by Robinson et al. (1987):

$$Kt \text{ DM (\%/h)} = 100 \times \left(\frac{\text{Dry matter intake (kg/d)}}{\text{DM ruminal content (kg)}} \right) / 24 \quad (9)$$

$$Kt \text{ DM (kg/h)} = \text{DM ruminal content (kg)} \times \left(\frac{Kt \text{ (\%/h)}}{100} \right) \quad (10)$$

4.2.5.4 Ruminal kinetics of fiber

For ruminal kinetics of the fiber determination, samples of feed (offered ration), ruminal content and feces collected from the 4 experimental periods were dried (55°C for 72 hours) and processed in a 2 mm sieve, for further determination of DM and NDF content. Amounts of 1.5 g of each sample were packed in polyethylene bags (F57-Ankom®) previously weighed. After the last day of the experiment (day 29, period 4), the bags were incubated for 288 hours in the rumen of the animals (VALENTE et al., 2011) fed with 60% of silage and 40% of concentrate. After removal of the samples from the rumen, the bags were washed until the water was clear and then dried at 45°C for 48 hours. The residues were analyzed for the iNDF content.

The consumption of NDF and iNDF was determined by multiplying the dry matter intake by the NDF and iNDF concentration of the experimental diets, respectively.

Ruminal mass rates of NDF and iNDF were estimated from the following equations:

$$RMf \text{ (kg)} = \frac{RMm \text{ (kg)} \times RNDF \text{ (\%)}}{100} \quad (11)$$

$$RMi \text{ (kg)} = \frac{RMm \text{ (kg)} \times RNDFi \text{ (\%)}}{100} \quad (12)$$

where:

RMf = Ruminal mass of NDF (kg);

RMm = Ruminal mass of DM (kg);

RNDF = Ruminal concentration of NDF (%);

RMi = Ruminal mass of FDNi (kg);

RNDFi = Ruminal concentration of FDNi (%);

Rates of NDF and NDFi ruminal kinetics were estimated from equation adapted from Oba & Allen (2003):

$$Kt \text{ NDF (\%/h)} = 100 \times \frac{\text{NDF intake (kg/d)}}{RMf \text{ (kg)}} / 24 \text{ h} \quad (13)$$

$$Kt \text{ NDFi (\%/h)} = 100 \times \frac{\text{NDFi intake (kg/d)}}{RMi \text{ (kg)}} / 24 \text{ h} \quad (14)$$

$$Kp \text{ NDF (\%/h)} = Kt \text{ NDF} - kd \text{ NDF} \quad (15)$$

where:

Kt NDF (%/h) = Rumen disappearance rate of NDF (%/h);

RMf = Ruminal mass of NDF (kg);

Kt NDFi (%/h) = Rumen disappearance rate of NDFi (%/h);

RMi = Ruminal mass of NDFi (kg);

Kp NDF = Ruminal passage rate of NDF (%/h);

kd NDF = Digestion rate of NDF (%). For this calculation, NDF degradability was used.

For calculation of the daily fecal flow of NDF (kg/d), pooled feces samples were collected between days 21 and 24 of each experimental period, following the distribution: day 21 - sampling at 18h00 and 14h00; day 22 - sampling at 08h00 and 04h00; day 23 – sampling at 10h00 and 06h00 and day 24 - sampling at 12h00 and 20h00, as indicated by

Sampaio et al. (2010). The samples were stored in freezer (-20°C) until the respective analyzes. The fecal flow rate was determined using the following equation:

$$F_{fNDF} \text{ (kg/d)} = \frac{NDF_{fI} \text{ (kg/d)}}{NDF_i \text{ (\%)}} \times 100 \quad (16)$$

where:

F_{fNDF} = Daily fecal flow of NDF (kg/d);

NDF_{fI} = Daily FDNi intake (kg/d);

NDF_i = Fecal concentration of NDFi (%).

4.2.6 Statistical analysis

Results were analyzed by the Statistical Analysis System (Version 9.4, SAS Inst., Inc., Cary, NC, USA 2015), after verifying residue normality by Shapiro-Wilk test (PROC UNIVARIATE) and variance homogeneity by Hartley test. Then, data were submitted to analysis of variance, which separated as causes of variation the effects of oil, square (monensin), period, as well as, animal within square. Data were analyzed using the MIXED procedure of SAS (Version 9.4, 2015), where the model included the effect of monensin, oil and the interaction between oil and monensin as fixed factors and the effects of period and animal within monensin as random factors. In the presence of oil effect, the means comparison was done by the Tukey test adjusted to 5% probability. The following statistical model was used:

$$Y_{ijkl} = \mu + O_i + M_j + (O_i \times M_j)_{ij} + P_k + A_l(M_j) + e_{ijkl}$$

where:

Y_{ijkl} = Observation related to Oil (i) + Monensin (j) + Oil (i) x Monensin (j) + Period (k) + Animal (l) inside Monensin (j) + random error associated to each observation (e_{ijkl});

μ = Average mean associated to the variable;

O_i = Fixed effect of oil i;

M_j = Fixed effect of monensin j;

$(O_i \times M_j)_{ij}$ = Interaction effect between oil (i) and monensin (j);

P_k = Random effect of period k;

$A_i(M_j)$ = Random effect of animal (i) inside monensin (j);

e_{ijkl} = Experimental random error associated to each observation.

4.3 RESULTS

4.3.1 Dry matter and water intake

Feeding SOY resulted in lower ($P=0.0451$) DMI (kg/d; % of BW or g/kg of MW) compared to control treatment (CONT). Inclusion of monensin in diets did not alter DMI. There was no significant effect ($P>0.05$) of oil source or monensin inclusion in diets for water intake (Table 17).

Table 17 - Effect of diet supplementation with vegetable oils and monensin on dry matter and water intake by Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
DMI, kg/d	9.41	8.96	9.80 ^a	9.05 ^{ab}	9.15 ^{ab}	8.73 ^b	0.3069	0.6909	0.0451	0.6233
DMIBW, %	1.98	1.85	2.03 ^a	1.89 ^{ab}	1.90 ^{ab}	1.84 ^b	0.0615	0.5484	0.0493	0.6216
DMI, g/kg de MW	91.9	86.3	94.7 ^a	87.7 ^{ab}	88.6 ^{ab}	85.5 ^b	2.4132	0.4495	0.0320	0.5271
WI, L/d	24.6	23.9	26.2	22.0	25.8	22.8	1.4618	0.8906	0.4681	0.2853
WI/DMI, L/kg	2.63	2.71	2.72	2.45	2.86	2.64	0.1538	0.7459	0.7010	0.1934
WIBW, %	5.08	4.94	5.24	4.58	5.44	4.78	0.2724	0.8427	0.5653	0.4775
WIMW, mL/kg de PM	236.8	230.7	246.9	213.1	252.9	222.1	12.205	0.8336	0.5283	0.4318

¹DMI: Dry matter intake (kg/d); DMIBW: Dry matter intake in relation to body weight (%); DMIMW: Dry matter intake per unit of metabolic weight (g/kg of MW); WI: Water ingestion (L/d); WI/DMI: Water intake in relation to dry matter intake (L/kg); WIBW: Water intake in relation to body weight (%); WIMW: Water intake in relation to metabolic weight (mL/kg of MW); ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statically differ by Tukey test ($P<0.05$).

4.3.2 Ingestive behavior

Mean time per event (MTE, min/event) in chewing ($P=0.0294$) was increased by 5.15% and 6.83% by the treatments CAN and SOY, respectively, when compared to the treatment CONT (Table 18).

No significant effect ($P>0.05$) of oil source inclusion in the diets was observed for the other variables of ingestive behavior (Idleness, Ruminating, Eating and Drinking) evaluated. Likewise, there was no effect ($P>0.05$) of monensin on any of the variables of ingestive behavior.

Table 18 - Effect of diet supplementation of vegetable oils and monensin on ingestive behavior in Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	M x O
Idleness										
TTA, min/d	743.6	672.8	723.1	715.6	676.9	717.3	12.371	0.0706	0.0809	0.9110
NE	21.19	21.13	21.63	20.63	21.50	20.88	0.5483	0.9595	0.9240	0.8813
ETTA, %	51.50	46.59	50.08	49.56	46.88	49.67	0.8567	0.0705	0.0811	0.9114
MTE, min/event	35.13	32.81	34.09	35.45	32.25	34.10	1.1470	0.3595	0.8398	0.8816
Ruminating										
TTA, min/d	475.6	493.1	470.0	471.3	501.9	494.4	11.717	0.4528	0.6863	0.5373
NE	16.19	15.00	16.50	14.63	16.13	15.13	0.5136	0.3316	0.5411	0.7722
ETTA, %	32.94	34.15	32.55	32.64	34.76	34.24	0.8114	0.4527	0.6857	0.5378
MTE, min/event	29.84	33.98	28.81	32.82	31.98	34.04	1.0108	0.1726	0.1645	0.6193
Eating										
TTA, min/d	219.1	260.0	228.8	239.4	246.9	243.1	9.2739	0.0820	0.8533	0.7709
NE	10.19	12.25	10.88	11.50	11.63	10.88	0.4725	0.1539	0.6839	0.4952
ETTA, %	15.17	18.01	15.84	16.58	17.10	16.84	0.6422	0.0820	0.8540	0.7717
MTE, min/event	22.26	21.51	21.97	21.28	21.68	22.61	0.7635	0.7647	0.9035	0.4277
Chewing										
TTA, min/d	682.3	753.1	698.8	710.6	748.8	712.7	12.017	0.0676	0.0735	0.9007
NE	26.38	27.50	27.38	26.13	27.75	26.00	0.6606	0.4455	0.6853	0.3129
ETTA, %	47.19	52.46	48.39	49.83	51.85	49.25	0.8322	0.0677	0.0578	0.8443
MTE, min/event	26.59	26.79	25.77 ^b	27.17 ^a	26.14 ^{ab}	27.66 ^a	0.4467	0.8854	0.0294	0.1154
Drinking										
TTA, min/d	19.06	19.66	24.30	18.77	19.38	15.00	2.2611	0.9385	0.3990	0.8974
NE	3.44	3.42	3.63	3.82	3.62	2.63	0.3965	0.9876	0.5968	0.8075
ETTA, %	1.32	1.32	1.60	1.29	1.34	1.04	0.1565	0.9962	0.4135	0.8575
MTE, min/event	5.52	4.71	5.03	4.38	5.52	5.54	0.2216	0.1573	0.4189	0.6690

¹TTA: Total time in activity (min/dia); NE: Numbers of events per day; ETTA: Events per total time in activity (%); MTE: Mean time per event (min/event); ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P < 0.05$).

The treatment CAN decreased ($P=0.0301$) the ingestion rate of NDF (kg/event) in 24.4% and the rumination rate of NDF (kg/min) in 22.2% in relation to control treatment (Table 19).

There was a reduction ($P=0.0297$) in chewing rate of DM, when expressed in kg/min when animals received SOY treatment (Table 19). For chewing rate of NDF, when expressed in kg/min, CAN and SOY treatments reduced this variable by 33.3%, in comparison to the control treatment. An effect was observed for this variable, when

expressed in min/kg, where chewing rate of NDF increased in 17.5% in CAN and 17.3% in SOY treatment, compared to control.

Ingestion, rumination and chewing rates were not affected ($P>0.05$) by monensin inclusion or not in diets. Also, no interaction effect (MxO) was observed for these variables.

Table 19. Effect of diet supplementation of vegetable oils and monensin on ingestion, rumination and chewing rates in Nellore cattle.

Variables	Monensin		Oil ¹				SEM ²	P-value ³		
	Without	With	CONT	CAN	SUN	SOY		M	O	M x O
Ingestion rate										
DM, kg/min	0.045	0.036	0.045	0.038	0.039	0.040	0.0018	0.1093	0.3935	0.7301
NDF, kg/min	0.018	0.015	0.018	0.015	0.016	0.016	0.0007	0.1099	0.2125	0.7288
DM, min/kg	23.42	29.54	23.08	27.28	27.22	28.34	1.3580	0.1178	0.3946	0.4303
NDF, min/kg	58.31	69.66	56.86	71.59	65.04	62.43	2.8466	0.2286	0.1083	0.9117
DM, kg /Event	0.99	0.75	0.97	0.82	0.85	0.86	0.0436	0.1007	0.2263	0.9501
NDF, kg /Event	0.40	0.31	0.41 ^a	0.31 ^b	0.36 ^{ab}	0.34 ^{ab}	0.0183	0.1574	0.0301	0.9790
Rumination rate										
DM, kg/min	0.021	0.019	0.021	0.019	0.019	0.019	0.0008	0.5227	0.1476	0.6942
NDF, kg/min	0.008	0.007	0.009 ^a	0.007 ^b	0.008 ^{ab}	0.008 ^{ab}	0.0003	0.6060	0.0270	0.8164
DM, min/kg	51.74	56.29	48.47	54.17	55.74	57.66	2.5279	0.5691	0.1609	0.9340
NDF, min/kg	128.8	140.0	119.4	142.2	133.2	142.9	6.3009	0.5690	0.0993	0.9369
DM, kg /Event	0.60	0.64	0.61	0.62	0.61	0.65	0.0330	0.6930	0.9579	0.9187
NDF, kg /Event	0.24	0.26	0.25	0.24	0.26	0.26	0.0134	0.6898	0.8812	0.9219
Chewing rate										
DM, kg/min	0.014	0.012	0.014 ^a	0.012 ^{ab}	0.013 ^{ab}	0.012 ^b	0.0004	0.2406	0.0297	0.5219
NDF, kg/min	0.006	0.005	0.006 ^a	0.004 ^b	0.005 ^{ab}	0.004 ^b	0.0002	0.2405	0.0089	0.5108
DM, min/kg	75.15	85.83	71.56 ^b	81.45 ^{ab}	82.96 ^{ab}	86.00 ^a	3.1956	0.3188	0.0359	0.5686
NDF, min/kg	187.1	213.6	176.3 ^b	213.8 ^a	198.2 ^{ab}	213.2 ^a	8.0078	0.3186	0.0143	0.5635
DM, kg /Event	0.37	0.34	0.37	0.35	0.35	0.35	0.0117	0.4232	0.6866	0.9691
NDF, kg /Event	0.15	0.14	0.15	0.13	0.15	0.14	0.0049	0.4285	0.2284	0.9820

¹CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ²SEM: Standard error of the mean; ³M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P<0.05$).

4.3.3 Percentage of particle size distribution and diet selection index

The table 20 shows that the diet selection index and the percentage of particle size distribution of leftovers (PPSD_L,%) did not show significant effect ($P>0.05$) of oil or monensin inclusion in diets. However, for the percentage distribution of particle size of total diet (PPSD_{TD},%) on fresh matter basis, a significant effect was observed for the 8mm

sieve. Lipids sources increased (5.79% for CAN and SUN; 6.20% for SOY) the number of particles of the total diet in this compartment compared to control. This observation was contrary to what was observed in the sieve 3 (box), where the number of particles was reduced (5.23% for CAN, 5.60% for SUN and SOY) in relation to control treatment.

Regarding diet selection index, for 19 mm sieve, selection against (index less than 1) was observed for all treatments with lipid source inclusion. However, for monensin inclusion, there was no selection (index equal to 1). For 8mm sieve and the box, absence of selection of feeds that were contained in the TMR was observed. These results are related to the absence of oil or monensin effect on the percentage of particle size distribution of leftovers.

Table 20 - Effect of diet supplementation with vegetable oils and monensin on diet selection index and the percentage distribution of particle size of total diet and leftovers of Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	M x O
Sieve 1 (Particle size - 19 mm)										
Selection index	0.83	1.00	0.90	0.95	0.91	0.93	0.0483	0.3868	0.8813	0.2062
PPSD _{TD} , %	4.26	4.48	4.12	4.38	4.58	4.41	0.1633	0.2391	0.3434	0.1688
PPSD _L , %	7.81	3.79	6.74	5.28	5.93	5.27	1.0976	0.3904	0.6310	0.1914
Sieve 2 (Particle size - 8 mm)										
Selection index	1.00	1.03	1.00	1.03	1.01	1.02	0.0078	0.3009	0.3725	0.6734
PPSD _{TD} , %	44.4	44.3	42.3 ^b	44.9 ^a	44.9 ^a	45.1 ^a	0.5111	0.9592	0.0151	0.6361
PPSD _L , %	42.9	35.6	38.2	38.2	41.2	39.5	1.9965	0.2990	0.5094	0.2627
Sieve 3 (Box, particle size < 8 mm)										
Selection index	1.03	0.98	1.03	0.98	1.00	1.00	0.0118	0.2528	0.0722	0.0722
PPSD _{TD} , %	51.4	51.2	53.5 ^a	50.7 ^b	50.5 ^b	50.5 ^b	0.5976	0.8203	0.0187	0.4725
PPSD _L , %	49.3	60.6	55.1	56.5	52.8	55.2	2.8482	0.3088	0.6550	0.1807

¹PPSD_{TD} (%): Percentage of particle size distribution of total diet; PPSD_L (%): Percentage of particle size distribution of leftover; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P < 0.05$).

4.3.4 Rumen kinetics variables

4.3.4.1 Rumen degradability

Crude protein soluble fraction (a) was reduced ($P = < 0.0001$) with the addition of lipid sources in the experimental diets compared to CONT diet. For neutral detergent fiber soluble fraction, CAN treatment reduced this variable by 44.0% in relation to CONT

treatment and did not differ from the other treatments with oil inclusion without monensin (Table 21).

Table 21 - Effect of diet supplementation with vegetable oils and monensin on rumen degradability of dry matter, crude protein and neutral detergent fiber in Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
DM variables (%)										
a, %	23.37	23.56	22.17	23.68	24.78	23.24	0.4819	0.8328	0.1087	0.9161
b, %	51.27	49.98	57.13 ^a	48.59 ^b	46.76 ^b	50.00 ^b	0.9477	0.3180	<0.0001	0.3172
c, h ⁻¹	0.054	0.052	0.039 ^b	0.055 ^a	0.059 ^a	0.060 ^a	0.0022	0.6380	<0.0001	0.4395
PD, %	74.64	73.54	79.31 ^a	72.28 ^b	71.54 ^b	73.24 ^b	0.7432	0.2269	<0.0001	0.0561
Ind, %	25.36	26.46	20.69 ^b	27.72 ^a	28.46 ^a	26.76 ^a	0.7432	0.2269	<0.0001	0.0561
ED 2, %/h	59.95	59.46	59.58	59.20	59.45	60.59	0.5200	0.4979	0.4279	0.5004
ED 5, %/h	49.25	48.87	47.01 ^b	49.00 ^{ab}	49.85 ^a	50.39 ^a	0.6248	0.6260	0.0170	0.7162
ED 8, %/h	43.46	43.13	40.79 ^b	43.36 ^{ab}	44.44 ^a	44.59 ^a	0.6429	0.6417	0.0028	0.7255
RD, %	47.35	53.58	46.05 ^b	50.57 ^{ab}	53.13 ^a	52.13 ^{ab}	1.2797	0.0401	0.0439	0.6709
CP variables (%)										
a, %	15.84	15.81	24.46 ^a	13.06 ^b	12.28 ^b	13.49 ^b	1.2900	0.9875	<0.0001	0.2371
b, %	71.25	69.76	66.91	71.11	71.03	72.96	1.4965	0.5907	0.4702	0.0352
c, h ⁻¹	0.062	0.059	0.039 ^b	0.069 ^a	0.064 ^a	0.072 ^a	0.0033	0.6434	0.0002	0.3003
PD, %	87.09	85.57	91.37	84.18	83.31	86.46	1.2655	0.5067	0.0799	0.1097
Ind, %	12.91	14.43	8.628	15.82	16.69	13.54	1.2655	0.5067	0.0799	0.1097
ED 2, %/h	67.18	67.59	65.65 ^b	68.04 ^{ab}	65.64 ^b	70.23 ^a	1.0066	0.7317	0.0365	0.8689
ED 5, %/h	53.16	53.43	51.54	54.16	51.35	56.13	1.1780	0.8735	0.0818	0.4515
ED 8, %/h	45.34	45.42	44.76	45.91	43.16	47.68	1.1819	0.9648	0.1741	0.3043
RD, %	52.57	52.65	55.79 ^a	52.71 ^a	48.89 ^b	53.04 ^a	0.8340	0.9392	0.0005	0.9553
NDF variables (%)										
a, %	11.59	10.76	11.77 ^a	6.595 ^b	12.38 ^a	13.97 ^a	0.7279	0.3370	<0.0001	0.3640
b, %	50.54	50.77	61.77 ^a	48.48 ^b	46.58 ^b	45.79 ^b	1.6930	0.8979	<0.0001	0.4350
c, h ⁻¹	0.027	0.023	0.020	0.025	0.025	0.029	0.0016	0.3020	0.1821	0.9396
PD, %	61.79	61.28	72.87 ^a	55.07 ^b	58.44 ^b	59.75 ^b	1.6031	0.7961	<0.0001	0.5481
Ind, %	38.21	38.72	27.13 ^b	44.93 ^a	41.56 ^a	40.25 ^a	1.6031	0.7961	<0.0001	0.5481
ED 2, %/h	38.33	36.96	40.76 ^a	32.88 ^c	36.76 ^b	40.17 ^a	0.8611	0.2846	<0.0001	0.0493
ED 5, %/h	27.57	26.05	28.03 ^{ab}	22.37 ^c	26.64 ^b	30.21 ^a	0.8639	0.1801	<0.0001	0.2553
ED 8, %/h	22.97	21.53	22.95 ^{ab}	17.88 ^c	22.39 ^b	25.78 ^a	0.8401	0.1560	<0.0001	0.4038
RD, %	27.05	25.84	30.46 ^a	21.51 ^c	25.33 ^b	28.48 ^a	0.8289	0.3386	<0.0001	0.1031

¹a: water-soluble and rapidly degradable fraction, b: potentially degradable fraction, c: rate of degradation per hour of the potentially degradable fraction; PD: potential degradability; Ind: Non-degradable fraction; ED: Effective degradability; RD: Real degradability; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P < 0.05$).

Dry matter and NDF potentially degradable fraction (b) decreased in all treatments with oil inclusion in 14.9%, 18.1% and 12.5% (DM) and 21.5%, 24.6% and 25.9% (NDF), respectively, by CAN, SUN and SOY, in relation to CONT treatment. However, crude protein potentially degradable fraction (b) was not affected by treatments ($P > 0.05$).

The addition of vegetable oils in diets (CAN, SUN and SOY) resulted in lower potential degradability (PD, %) of DM (8.9%, 9.8% and 7.7%, respectively) and NDF (24.4%, 19.8% and 18.0%, respectively), whereas the non-degradable fraction (Ind, %) was increased in relation to the CONT treatment. Potential degradability (PD, %) of crude protein and its non degradable fraction (Ind, %) was not affected by vegetable oils inclusion in diets. For all these variables, there was no significant effect ($P > 0.05$) of monensin addition.

Regarding the ED (%) of DM (5 %/h and 8 %/h), these variables were increased by SUN and SOY treatments when compared to treatment without addition of lipid source (control). Real degradability (RD, %) of DM was affected by oil and monensin factors, where SUN treatment increased RD of DM by 15.4% (vs. CONT). When monensin was added in the diet an increase of 13.1% in RD of DM was observed in relation to non-addition of monensin. In relation to the RD (%) of CP, SUN treatment reduced this variable in 12.4%, in relation to the treatments CONT. The addition of monensin in diet had no significant effect ($P > 0.05$) in this variable.

Real degradability of NDF was reduced by treatments CAN (29.4%) and SUN (16.8%) when compared to control treatment. Similar effect was observed for effective degradability (ED, %/h) of NDF for passage rates of 2 %/h; 5 %/h; 8 %/h. The addition of monensin in diet had no significant effect ($P > 0.05$) in these variables.

4.3.4.2 Ruminal dynamics, disappearance rate and dry matter rumen passage rate

No significant effect ($P > 0.05$) of oil or monensin inclusion in diets was observed in relation to liquid, solid and total ruminal mass when expressed in kg or % of BW (Table 22).

Ruminal disappearance rate of dry matter (%/h) decreased ($P = 0.0029$) with the addition of vegetable oils in 12.9%, 10.8% and 12.4% in CAN, SUN and SOY treatments, respectively, compared to CONT treatment. However, ruminal passage rate of dry matter was not affected ($P > 0.05$) by the addition of different sources of oil in diets.

With monensin inclusion in diets, there was no significant difference ($P>0.05$) for Kt of DM. However, Kp (%/h) decreased ($P=0.0018$) in 40.9% when monensin was added in the diet.

Table 22 - Effect of diet supplementation with vegetable oils and monensin on rumen dynamics, disappearance and rumen passage rate of dry matter in Nellore cattle.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
DM ruminal content, %	12.9	13.2	12.8	13.2	13.0	13.2	0.1566	0.6228	0.6677	0.3872
Liquid mass, kg	34.8	38.1	36.1	35.4	36.9	37.4	1.4850	0.6183	0.5469	0.7813
Liquid mass, % of BW	7.11	7.70	7.39	7.16	7.52	7.55	0.1563	0.1685	0.4614	0.8014
Solid mass, kg	5.18	5.73	5.30	5.34	5.51	5.67	0.2216	0.5699	0.4844	0.5085
Solid mass, % of BW	1.06	1.15	1.08	1.09	1.13	1.11	0.0263	0.2279	0.5100	0.3744
Total mass, kg	39.9	43.8	41.4	40.7	42.4	43.2	1.6960	0.6110	0.5150	0.7413
Total mass, % of BW	8.17	8.86	8.47	8.25	8.65	8.69	0.1786	0.1701	0.4570	0.7485
Kt DM, %/h	8.41	7.22	8.59 ^a	7.48 ^b	7.66 ^b	7.52 ^b	0.2576	0.1571	0.0029	0.0579
Kt DM, kg/h	0.43	0.41	0.45	0.39	0.41	0.42	0.0149	0.7113	0.0923	0.7669
Kp DM, %/h	6.17	3.61	6.06	4.80	3.89	4.81	0.4264	0.0018	0.2290	0.5856

¹Kt: Ruminal disappearance rate of dry matter; Kp (%/h): Ruminal passage rate of dry matter; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P<0.05$).

4.3.4.3 Rumen kinetics of fiber

Feeding SOY resulted in lower ($P=0.0360$) NDF intake (kg/d) than control treatment (CONT). While for iNDF intake, CAN treatment led to a greater reduction (14.4%) in this variable compared to control treatment (Table 23).

The Kt (%/h) of NDF was reduced ($P=0.0072$) by SUN and SOY treatments by 16.7% and 17.9%, respectively. However, Kp and Kd of NDF were not affected ($P>0.05$) by the addition of oil or monensin in diets. Monensin addition also did not influence the Kt of NDF.

Regarding Kt of iNDF (%/h), CAN reduced this variable by 16.0% compared to CONT treatment. Similar effect was observed for daily fecal flow, which was reduced by 13.9% by CAN treatment compared to control.

Table 23 - Effect of diet supplementation with vegetable oils and monensin on rumen kinetics of fibre in Nellore cattle.

Variables ¹	Monensin		Óleo ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
NDF ruminal content, %	65.3	64.3	63.3	65.4	65.9	64.6	0.9011	0.3977	0.3771	0.0847
iNDF ruminal content, %	33.6	36.6	34.8	35.4	35.2	34.9	0.5578	0.1492	0.9454	0.3105
NDFI, kg/d	2.97	2.93	3.17 ^a	2.93 ^{ab}	2.87 ^{ab}	2.82 ^b	0.0898	0.8904	0.0360	0.3894
iNDFI, kg/d	1.30	1.28	1.39 ^a	1.19 ^b	1.30 ^{ab}	1.27 ^{ab}	0.0392	0.8856	0.0157	0.4204
Ruminal mass of NDF, kg	3.37	3.68	3.36	3.47	3.62	3.65	0.1477	0.6124	0.3652	0.1613
Ruminal mass of iNDF, kg	1.75	2.10	1.85	1.91	1.95	2.00	0.0925	0.3783	0.6573	0.8974
Kt NDF, %/h	3.81	3.38	4.07 ^a	3.59 ^{ab}	3.39 ^b	3.34 ^b	0.1224	0.2944	0.0072	0.2592
Kt iNDF, %/h	3.27	2.62	3.25 ^a	2.73 ^b	2.96 ^{ab}	2.84 ^{ab}	0.1309	0.1720	0.0322	0.7959
Kp NDF, %/h	1.51	1.47	2.31	1.07	1.41	1.20	0.1973	0.9417	0.0818	0.5418
Kd NDF, %/h	2.66	2.33	2.01	2.52	2.51	2.95	0.1663	0.3020	0.1821	0.9396
Daily fecal flux, kg/d	4.19	4.08	4.48 ^a	3.86 ^b	4.13 ^{ab}	4.08 ^{ab}	0.1369	0.8031	0.0460	0.9189

¹NDFI: Neutral detergent fiber intake; iNDFI: Indigestible neutral detergent fiber intake; Kt NDF: Ruminal disappearance rate of neutral detergent fiber; Kt iNDF: Ruminal disappearance rate of indigestible neutral detergent fiber; Kp NDF: Ruminal passage rate of neutral detergent fiber; Kd NDF: Ruminal digestion rate of neutral detergent fiber; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil;

^{ab}Different letters in the same line statistically differ by Tukey test ($P < 0.05$).

4.4 DISCUSSION

The decrease in DMI, after inclusion of a source of ω -3 PUFA in cattle diet, such as soybean oil, was associated to greater chewing time (min/event) and chewing rate of DM and NDF (min/kg). This result was also related to chewing rate of DM and NDF when expressed in kg/min, leading to a reduction in the amount of chewed fraction per time. These effects were due to the reduction in the degradation of DM and potentially degradable NDF (fraction b), as well as, in the potential degradability (PD) of each of the components (DM and NDF), which led to a decrease in ruminal disappearance rate. All these effects were closely related to Kt (%/h) of DM and NDF which also decreased with lipid sources addition in diets.

Rogers & Davis (1982) indicate that the decrease in DMI when lipid sources are included in diets is due to increased retention time of feed in the rumen. Accordingly, the reduction in DMI, explained by the decrease in ruminal emptying observed by the rate of disappearance of both DM and NDF, may lead to a satiety effect, either due to a metabolic effect caused by long chain fatty acids (CHILLIARD et al., 1993) or by a stimulus to rumen mechanoreceptors, caused by ruminal fill, mainly due to fiber degradation

reduction. What was observed in the present experiment agrees with Beauchemin et al. (2007), who mention that the reduction in DMI was probably due to a 30% decrease in NDF digestion, when a source of PUFA, such as sunflower, is included in cattle diets. This could have contributed to rumen fill. Moallem et al. (2007) reported that the degree of fatty acid insaturation had a marked effect on DMI. Fatty acids with higher number of unsaturations lead to a greater depression on DMI, and this may be related to the possible effect of toxicity on ruminal microorganisms, resulting in lower fiber degradability in the rumen. This fact was observed in the present study in soybean oil treatment, which is a highly unsaturated oil source. According to Allen (2000), the mechanisms by which lipid supplementation in ruminant diet result in inhibition of DMI are associated to changes in ruminal fermentation, intestinal motility, intestinal hormone release and limited capacity of ruminants in fatty acids oxidation.

Although a significant effect of MUFA source (canola oil) on DMI was not observed, this oil source had a negative influence on ingestion (kg/event) and rumination (kg/min) rate of the NDF, with a decrease in chewing rate (kg/min) of this fraction. These results indicate that there is a marked effect on intake, rumination and chewing rates of MUFA (canola) and PUFA (soybean), which present high levels of n-3, as well as, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The results of the present experiment show that the inclusion in diets of a source rich in PUFA, such as soybean, in the proportion of 3.5% of DM, negatively affects DMI of cows. The magnitude of this effect depends on the lipid source: the degree of lipids insaturation, the formation of carboxylate salts and physical associations with the surface of feed particles and microorganisms (UEDA et al., 2003; PINOS-RODRIGUEZ, 2009). The effect tends to be greater when the diet forage is corn silage (MARTÍNEZ et al., 2013).

Jenkins & McGuire (2006) indicate that the main effect of lipid inclusion in diets on DMI depression are related to changes in ruminal fermentation. Specifically, a reduction in fiber degradability in the rumen results in increased NDF retention time, which results in increased rumen fill.

The absence of monensin effect on DMI was associated to the absence of monensin effect on degradability variables. Russell and Strobel (1989) showed in *in vivo* experiments that with monensin supplementation, fiber digestion remained unchanged, but there was a decrease in the passage rate of solid material from the rumen to the gut. In this way, the fibrous particle remained a longer time in rumen environment, extending fermentation time, like what was observed in the present experiment with monensin addition.

For the percentage of particle distribution, the increased amount of particles of total diet that contained fatty acids in the form of oil, in 8 mm sieve and the reduction of these particles in the box (sieve 3, particles <8mm) is more associated to the diet structure. Although all diets were composed of a TMR with 60% of forage and 40% of concentrate, the addition of oil directly to the diets did not allow the passage of the particles to the sieve 3, by the effect of compaction and coating generated by the oil to the other ingredients in the diet.

In relation to ruminal degradability variables, the reduction observed for the degradation of the protein fraction (a) was due to lower crude protein solubility generated by the addition of vegetable oils in the diet.

The present experiment shows that with the addition of 3.5% DM of lipid sources (MUFA and PUFA) in oil form, there is a decrease in the potentially degradable fraction (b) and the Pd (%) of DM and NDF, which is directly related to the increase of the non-degradable fraction (Ind,%) of these components. It is well known that the addition of lipids in cattle diets can inhibit fiber digestion in the rumen (MACZULAK et al., 1981; JENKINS, 1993), due to the inhibition of microorganisms that degrade fiber by the toxic effect that lipids exert on them (PALMQUIST; MATTOS, 2006), as well as the physical barrier (coating action) that lipids create by involving feed particles (JENKINS; MCGUIRE, 2006; PALMQUIST; MATTOS, 2006), which limits the colonization of the particles by microorganisms. This step is necessary for hydrolysis to occur through digestive action of bacterial enzymes in potentially digestible NDF sites (PALMQUIST; MATTOS, 2006). As a result of these mechanisms, which agree with the findings of the present study, there is a reduction in the digestion of the fibrous fraction because of an increase in the indigestible fraction (Ind, %) and a reduction of the potentially digestible fraction of lipids. The reduction of DM and fiber degradability observed was a result of the lower disappearance rate (K_t , %/h) of these components in the rumen, which may reduce fermentation efficiency by providing less nitrogen and energy for microbial growth (WALDO, 1986). It is believed that the degree of lipid source unsaturation above the saturation capacity of microorganisms and the rate of release of these into the rumen are positively associated with a decrease in ruminal degradation (NRC, 2001; SILVA et al., 2007), besides the quantity added in diet. Therefore, the usual recommendation is that the total fat content of the diet does not exceed 50 g/kg DM to avoid depressions in NDF intake and digestibility, which, in turn, would reduce the benefit of a higher energy density in the diet by lipids addition (PALMQUIST; JENKINS, 1980). However, Palmquist (1989)

and Jenkins (1993) suggested the threshold of 70 g/kg of DM and above these values would reduce both NDF digestion and DMI. In the present study, an amount of oil of 35 g/kg DM was used, which was lower than that indicated by other studies and resulted in decreased ruminal degradation.

Regarding CP, despite no treatment effect was observed for PD (%), as well as for the non-degradable fraction (Ind, %) of this component, when it was expressed as RD (%), PUFA present in SUN treatment reduced the degradation of this fraction. It should be noted that there is no experimental evidence of lipids action on rumen nitrogen degradability, although some experiments have shown increased degradability. Factors of variation of proteolytic activity of bacteria are not clearly established (BRODERICK et al., 1991). Therefore, the only factor that could explain an increase in protein degradation is probably the decrease in protozoa number subsequent to lipid addition. A fact that involves an increase in bacterial biomass and, therefore, a globally larger proteolytic activity (USHIDA et al., 1991). However, this fact was not observed.

The present experiment showed that PUFAs increased ED (5 %/h and 8 %/h) and RD (%) of DM. This indicates that a source high in omega 6 (n-6), such as sunflower, does not have a negative behavior in ruminal degradation, an effect that is generally expected to occur with the addition of PUFA. In this sense, sources rich in linoleic acid resulted in improvement of the RD of DM and decreased of the negative effect that lipids inclusion in diets normally generate. This effect presents sunflower oil, as a feed source with great potential in ruminant feeding, as it does not limit the use of dietary energy. Moreover, in the present experiment, it was possible to verify that the positive effect of sunflower oil on DM degradation is directly attributed to the lipid source itself and not to other parameters, since sunflower oil inclusion did not negatively affect DMI or feed passage rate.

Jenkins & Palmquist (1984) indicated that lipids addition in cattle diets, in the form of calcium salts of fatty acids, promoted a slight increase in DM and NDF ruminal digestion, when compared to a control diet.

The increase of RD of DM when monensin is included in the diet of animals has been described by Schelling (1984), who indicates that ionophores improve the digestion of feed particles. Another indirect contribution of the ionophore on feed particles digestion is to inhibit the negative coating effect exerted by oils, and which may have occurred, is that monensin decreases the concentration of lactate in the rumen, thereby limiting the pH drop in this environment, thus providing better conditions for the development of cellulolytic bacteria (RUSSEL; STROBEL, 1989).

In relation to the passage rate of DM (K_p , %/h), which was not affected by lipid treatments, some studies indicate that the inclusion of long chain fatty acids does not alter the K_p of DM (CHALUPA et al., 1986; COSTA, 2008; BARLETTA, 2014). Other studies mention that the addition of soybean oil leads to a decrease in the passage rate of DM, and that this effect is more pronounced in the forage than in the concentrate fraction.

Sodium monensin supplementation resulted in 40.9% reduction in K_p (%/h) of DM. Some studies have indicated that monensin may reduce rumen passage rate by 44% in steers fed low-quality grasses and reduced the passage rate in the whole gastrointestinal tract by 10% in grazing cattle (LEMENAGER et al., 1978). It is also worth to mention that monensin promotes reduced DMI, followed by changes in feed passage rate (MACHADO; MADEIRA, 1990), as well as improvement in diets digestibility (WEDEGAERTNER; JOHNSON, 1983). In a study conducted by LINNEEN et al. (2015), where steers were supplemented with monensin, the particle passage rate was reduced by approximately 42%, compared to the mean passage rate obtained for control treatment. The authors suggested that the influence of monensin on passage rate was largely due to the reduction of DMI among steers. Pond & Ellis (1979) and Pond et al. (1980) also verified that particle passage rate was decreased in cattle supplemented with monensin and fed high forage diets.

Cecava & Parker (1993), working with steers fed corn silage and concentrate (62:38 ratio), observed a passage rate of control diet marked with ytterbium of 6.9 %/h. Burger et al. (2000) worked with similar forage:concentrate ratio, but with soybean meal inclusion in diets, observed a passage rate of 6.8 %/h, using chromium oxide, as a marker. Cavalcante et al. (2004) described a passage rate of 4.4 %/h in cattle fed 60% corn silage and 40% concentrate with chromic oxide infusion. These results were similar to those obtained in the present experiment. On the other hand, Barletta (2014) evaluated lipid sources such as soybean oil, soybean grain and calcium salts of fatty acids and observed passage rate values of 3.2, 3.1 and 3.0 %/h, respectively. These values were lower than those found in the present experiment.

For passage rate of NDF (K_p NDF, %/h), Bettero (2015) evaluated lipid sources in the form of soybean oil, soybean grain and calcium salts of fatty acids and observed values of K_p NDF of 1.7, 1.4 and 2.1 %/h, respectively. These values were close to those obtained in the present research. Barletta (2014) evaluated the same treatments and found values of 2.1, 2.1 and 1.9 %/h, respectively, which were higher than those found in this study. It

should be noted that, these studies used the same technique of evaluation of fiber ruminal kinetics (internal marker).

The increase in rumen non-degradable fraction (Ind, %) of DM and NDF, led to a decrease in disappearance rate K_t (%/h) of DM and NDF by the addition of lipid sources, considering that PUFA influences the fiber component. Thus, feed particle remained greater time in ruminal environment, increasing fermentation time. Fatty acids can also adhere to feed particle, creating a physical barrier to microorganisms' action and microbial enzymes, impairing feed degradation (JENKINS, 1993; BALIEIRO NETO; MELLOTI, 2007).

Studies indicate that lipid inclusion in ruminant diet influences DM degradability. Palmquist & Jenkins (1980) suggest a tolerance of 4 to 5% of lipid supplementation in order to not affect DM degradability. On the other hand, in a study carried out by Messana et al. (2012), who evaluated different lipid inclusion levels (2, 4 and 6% of DM), DM degradability of soybean meal was higher when the animals received 6% lipid in the diet. However, for potential degradability (PD) evaluated, values were higher for treatment with 2% of lipids inclusion in relation to treatments with 4 or 6% of lipids inclusion in the diet. A similar effect was found by Jacob et al. (2012) when 6% of sunflower and soybean oil were included in diets. Dry matter, NDF, ADF, and hemicellulose degradability were reduced in these treatments compared with control treatment, for an *in vitro* evaluation.

By data reported in literature, it is important to note that ruminal fermentation and DM and nutrients degradation is compromised by 6% of lipid sources inclusion level in diets (JACOB et al., 2012; MESSANA et al. , 2012), since this level of inclusion may modify the microbial population or inhibit its activity (DEVENDRA; LEWIS, 1974).

Regarding monensin supplementation for DM and nutrients degradability, literature indicates no consistency of the influence of this ionophore on these variables. However, monensin supplementation showed nutrients digestibility reduction in the rumen in some studies with cattle and sheep (OWENS et al., 1978; SIMPSON, 1980; MUNTIFERING et al., 1981; ROGERS et al., 1991). Osborne et al. (2004) did not observe effect of monensin on fiber rumen degradability. In contrast, Haimoud et al. (1995) observed that monensin supplementation altered nutrients digestion in the diet, reducing fiber ruminal digestibility. According to what was exposed above, the reasons for these discrepancies between studies may be related to differences in monensin levels of inclusion in diets and the interactions between intake, feed composition and monensin (MUTSVANGWA et al., 2002).

4.5 CONCLUSION

Source of omega-3 polyunsaturated fatty acids, such as soybean oil at an inclusion level of 3.5% of DM, results in a higher real degradability of CP and NDF, without compromising feed passage rate. This is a positive effect, different from what is usually observed by lipids inclusion in diets, due to coating of feed particles.

This study allowed us to verify that MUFA source present in canola oil or omega 6 PUFA sources present in sunflower oil reduce the effective and real degradability of NDF.

The addition of monensin in cattle diet, at a dose of 32 mg / kg DM, increases the real DM degradability by 13%, however, it reduces the passage rate of this fraction. This demonstrates that the mechanism of action of monensin is associated to an improvement in feed particles digestion as a consequence of a reduction in feed passage rate, which shows that the processes of digestion and passage act simultaneously and competitively on digesta removal from the rumen.

The present study indicates that the combination of monensin with sources of vegetable oils does not result in either synergistic, additive or antagonistic effect.

REFERENCES

AFRC (Agricultural and Food Research Council). **Energy and protein requirements of ruminants**. CAB International, Wallingford, UK. 1993.

ALLEN, M.S. Effects of diet on short-term regulation of feed intake by lactating dairy cattle, **Journal of Dairy Science**, v.83, n.7, p.1598-1624, 2000.

AOAC International, 2000. **Official Methods of Analysis of AOAC International**. 17th Edition. Gaithersburg, MD (USA)

AOCS. AMERICAN OIL CHEMISTS SOCIETY. **Official Method Am 5-04, Rapid determination of oil/fat utilizing high temperature solvent extraction**. 5 ed., The American Oil Chemists Society, Urbana, IL, USA, 2005.

BALIEIRO NETO, G.; MELOTTI, L. Efeitos de níveis de sebo sobre a degradabilidade in situ do farelo de soja e do feno de Tifton (*Cynodon dactylon* (L.) Pers.) em vacas secas, **Brazilian Journal of Veterinary Research and Animal Science**, v. 44, n.4, p. 243-253, 2007.

BARLETTA, R.V. **Avaliação da cinética ruminal e fluxo abomasal de ácidos graxos em vacas leiteiras suplementadas com fontes lipídicas**. 2014. Tese (Doutorado em Nutrição e Produção Animal) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2014.

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

BETTERO, V.P. **Fontes de ácidos graxos insaturados na alimentação de vacas leiteiras no período seco**. 2015. xiv, 133 p. Tese (doutorado) - Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Agrárias e Veterinárias, 2015.

BRODERICK, G.A.; WALLACE, R.J.; ORSKOV, E.R. Control of rate and extent of protein degradation. In: **Physiological Aspects of Digestion and Metabolism in Ruminants** (Tsuda T, Sasaki Y, Kawashima R, eds) Academic Press, London, p.541-592, 1991.

BÜRGUER, P.J.; PEREIRA, J.C.; COELHO DA SILVA, J.F.; CAMPOS, S.; VALADARES FILHO, S.C.; CECON, P.R.; PEREIRA J.C.; PEREIRA, B.S. Taxas passagem e cinética da degradação ruminal em bezerros holandeses alimentados com dietas contendo diferentes níveis de concentrado. **Revista Brasileira de Zootecnia** v.29, p.225, 2000.

CAVALCANTE, A.C.R.; PEREIRA, O.G.; VALADARES FILHO, S.C. et al. Dietas contendo silagem de milho (*Zea mays* L.) e feno de capim-Tifton 85 (*Cynodon spp.*) em diferentes proporções para bovinos. **Revista Brasileira de Zootecnia**, v.33, n.6, p.2394-2402, 2004.

CECAVA, M.J.; PARKER, J.E. Intestinal supply of amino acids in steers fed ruminally degradable and undegradable crude protein sources alone and in combination. **Journal of Animal Science**, v.71, p.1596–1605, 1993.

CHALUPA, W.; VECCHIARELLI, B.; ELSER, A.E. et al. Ruminal fermentation in vivo as influenced by long-chain fatty acids. **Journal of Dairy Science**, v.69, p.1293-1301, 1986.

CHILIBROSTE, P.; TAMMINGA, S.; BOER, H.; GIBB, M.J.; den DI JJEN, G. Duration of regrowth of ryegrass (*Lolium perenne*) effects on grazing behaviour, intake, rumen fill and fermentation of lactating cows. **Journal Dairy Science**, v. 83, n.5, p.984-995, 2000.

CHILLIARD, Y.; DOREAU, M.; GAGLIOSTRO, G.; ELMEDDAH, Y. Addition de lipides protégés (encapsulés ou savons de calcium) à la ration de vaches laitières, Effets sur les performances et la composition du lait, **INRA Prod Anim**, v.6, p.139-150, 1993.

Costa M.G. **Diets with diferentes lipids sources for dairy cow**. (in Spanish). Ph.D. Thesis – Animal Science – UFV, ViçosaMG, pp. 119. 2008.

CZERKAWSKI, J.W. **An introduction to rumen studies**, Oxford: Pergamon International Library of Science, Technology, Engineering and Social Studies, p.236, 1986.

DEVENDRA, C.; LEWIS, D. The interaction between dietary lipids and fibre in the sheep, **Animal Production**, v.19, p.67, 1974.

ELLIS, W.C.; DELANEY, D.S. Intraruminal responses to monensin in cattle grazing forages, **Beef Cattle Research in Texas**, 1982, Texas Agr, Exp, Sta, p.84, 1982.

FERLAY, A.; DOREAU, M. Influence of method of administration of rapeseed oil in dairy cows. 1. Digestion of nonlipid components. **Journal of Dairy Science**, v.75, p.2320–2327, 1992.

FOLCH, J.; LEES, M.; SLOANE-STANLEY, G. H. A simple method for the isolation and purification of total lipides from animal tissues. **The Journal of Biological Chemistry**, Baltimore, v. 226, p. 497-509, 1957.

GARRETT, W.N. Energy utilization by growing cattle as determined in 72 comparative slaughter experiments. **Energy metabolism**. Proc. Symp., v.26, p.3–7, 1980.

HAIMOUD, A.D.; VERNAY, M.; BAYOURTHE, C. et al. Avoparcin and monensin effects on the digestion of nutrients in dairy cows fed a mixed diet. **Canadian Journal of Animal Science**, v.75, n.3, p.379-385, 1995.

HEINRICHS, J.; KONONOFF, P. In evaluating particle size of forages and TMRs using the Penn State Particle Size Separator, **Dairy and Animal Science**, p.1-14, 1996.

IKWUEGBU, O.A.; SUTTON, J.D. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep, **British Journal of Nutrition**, v.48, p.365, 1982.

JACOB, A.B.; BALAKRISHNAN, V.; KATHIRVELAN, C. Effect of amount and source of vegetable oils in a high fibrous cattle diet on in vitro rumen fermentation, nutrient degradability and rumen cis-9, trans-11 CLA concentration. **Journal of Applied Animal Research**, v.40, p.148-153, 2012.

JENKINS, T.C. Lipid metabolism in the rumen. **Journal of Dairy Science**, v.76, p.3851-3863, 1993.

JENKINS, T.C.; MCGUIRE, M.A. Major advances in nutrition: impact on milk composition, **Journal of Dairy Science**, v.89, p.1302-1310, 2006.

JENKINS, T.C.; PALMQUIST, D.L. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. **Journal of Dairy Science**, v.67, p.978, 1984.

KRAMER, J.K.G.; FELLNER, V.; DUGAN, M.E.R. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. **Lipids**, Heidelberg, v. 32, p. 1219-1228, 1997.

LEMENAGER, R.P.; OWENS, F.N.; SHOCKEY, B.J.; LUSBY, K.S.; TOTUSEK, R. Monensin effects on rumen turnover rate, twenty-four hour VFA pattern, nitrogen components and cellulose disappearance, **Journal of Animal Science**, v.47, p.255, 1978.

LEONARDI, C.; ARMENTANO, L.E. Effect of Quantity, Quality, and Length of Alfalfa Hay on Selective Consumption by Dairy Cows, **Journal of Dairy Science**, v.86, p.557–564, 2003.

LINNEEN, S.K.; HARDING, A.R.; SMALLWOOD, M.T. et al. In vivo ruminal degradation characteristics and apparent digestibility of low-quality prairie hay for steers consuming monensin and Optimase, **Journal of Animal Science**, v.93, n.8, p.3941–3949, 2015.

MACHADO, P.F.; MADEIRA, H.M.F. Manipulação de nutrientes em nível de rúmen - efeitos do uso de ionóforos. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE Zootecnia, 1990, Piracicaba. **Anais...** Piracicaba: Fundação de Estudos Agrários Luiz de Queiroz, 1990. p.79–96.

MACZULAK, A.; DEHORITY, E.B.A.; PALMQUIST, D.L. Effects of long-chain fatty acids on growth of rumen bacteria, **Applied and Environmental Microbiology**, v.42, p.856, 1981.

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 Dairy Science**, v.85, n.5, p.1165-1175, 2002.

MARTÍNEZ, M.A.L.; PÉREZ, H.M.; PÉREZ, A.L.M. et al. Fat addition in the diet of dairy ruminants and its effects on productive parameters. **Revista Colombiana de Ciencias Pecuarias**, v.26, n.2, p.69-78, 2013.

MESSANA, J.D. et al. Effects of different lipid levels on protozoa population, microbial protein synthesis and rumen degradability in cattle. **Acta Scientiarum. Animal Sciences**, v. 34, n. 3, p.279-285, 2012.

MOALLEM, U.; KATZ, M.; ARIELI, A.; LEHRER, H. Effects of peripartum propylene glycol or fats differing in fatty acid profiles on feed intake, production, and plasma metabolites in dairy cows. **Journal of Dairy Science**, v.90, p.3846-3856, 2007.

MORRIS, F.E.; BRANINE, M.E.; GALYEAN, M.L.; HUBBERT, M.E.; FREEMAN, A.S.; LOFGREEN, G.P. Effect of rotating monensin plus tylosin and lasalocid on performance, ruminal fermentation, and site and extent of digestion in feedlot cattle, **Journal of Animal Science**, v.68, p.3069–3078, 1990.

MUNTIFERING, R.B.; THEURER, B.; NOON, T.H. Effects of monensin on site and extent of whole corn digestion and bacterial protein synthesis in beef steers. **Journal of Animal Science**, v.53, p.1565-1573. 1981.

MUTSVANGWA, T.; WALTON, J.P.; PLAIZIER, J.C.; DUFFIELD, T.F.; BAGG, R.; DICK, P.; VESSIE, G.; MCBRIDE, B.W. Effects of a monensin controlled-release capsule or premix on attenuation of subacute ruminal acidosis in dairy cows. **Journal of Dairy Science**, v.85, p.3454–3502, 2002.

NRC, NATIONAL RESEARCH COUNCIL. 2000. **Nutrient Requirements of Beef Cattle**: Seventh Revised Edition: Update 2000. Washington, DC: The National Academies Press. 248 p.

NRC. NATIONAL RESEARCH COUNCIL, **Nutrient requirements of dairy cattle**, 7th ed, Washington: National Academies Press, 2001, 381p

OBA, M.; ALLEN, M.S. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. **Journal of Dairy Science**, v.86, p.184-194, 2003.

ØRSKOV, E.R.; DEB HOVELL, F.D.; MOULD, F. The use of the nylon bag technique for evaluation of feedstuffs, **Tropical Animal Production**, v.5, n.3, p.195-213, 1980.

ØRSKOV, E.R.; McDONALD, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. **Journal Agricultural Science**, v.92, n.1, p.499-503, 1979.

OSBORNE, J. K.; MUTSVANGWA, T.; ALZAHAL, O.; DUFFIELD, T.F.; BAGG, R.; DICK, P.; VESSIE, G.; MCBRIDE, B.W. Effects of monensin on ruminal forage degradability and total tract diet digestibility in lactating dairy cows during grain-induced subacute ruminal acidosis. **Journal of Dairy Science**, v.87, p.1840-1847, 2004.

OWENS, F.N.; SHOCKEY, B.; FENT, R.W.; RUST, S.R. Monensin and abomasal protein passage of steers. **Journal of Animal Science**, v.47 (Suppl. 1), p.114 (Abstr.), 1978.

PALMQUIST, D. L. Suplementação de lipídio para vacas em lactação. In: Anais do Simpósio sobre Nutrição de Ruminantes. **Fundação de Estudos Agrários “Luiz de Queiroz”**, Piracicaba, p.11-25, 1989.

PALMQUIST, D.L.; JENKINS, T.C. Fat in lactation rations: review, **Journal of Dairy Science**, v.63, p.1, 1980.

PALMQUIST, D.L.; MATTOS, W.R.S. Metabolismo de lipídeos. In: **Nutrição de ruminantes**. Berchielli, T. T., ed. FUNEP, Jaboticabal, p.287-310, 2006.

POND, K.R.; ELLIS, W.C. The effects of monensin on intake, digestibility and rate of passage in cattle grazing coastal bermuda pasture. **Journal of Animal Science**, v.49 (Suppl. 1), p.32, 1979.

POND, K.R.; ELLIS, W.C.; TELFORD, J.P. Monensin effects on intake digestibility and rate of passage of ryegrass grazed by cattle. **Journal of Animal Science**, v.51 (Suppl. 1), p.53, 1980.

POND, K.P.; ELLIS, W.C. Effects of monensina on fecal output and voluntary intake of grazed coastal Bermudagrass, Beef Cattle Research in Texas, 1981, **Texas Agr, Exp, Sta**, p 31, 1981,

RICHARDSON, L.F.; RAUN, A.P.; POTTER, E.L.; COOLEY, C.O.; RATHMACHER, R.P. Effect of monensin on ruminal fermentation in vitro and in vivo. **Journal of Animal Science**, v.58, p.194-202, 1984.

ROBINSON, P.H.; TAMMINGA, S.; VAN VUUREN, A.M. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. **Livestock Production Science**, v.7, pp.37-62, 1987.

ROGERS, J. A.; DAVIS, C. L. Rumen volatile acid fatty production and nutrient utilization in steers fed a diet supplemented with sodium bicarbonate and monensin. **Journal of Dairy Science**, Champaign, v. 65, n. 6, p. 944-952, 1982.

ROGERS, M.; JOUNANY, J.P.; THIVEND, P.; FONTENOT, J.P. Comparative effects of feeding and duodenal infusion of monensin on digestion in sheep. **Canadian Journal of Animal Science**, v.71, p.1125–1133, 1991.

RUSSEL, J.B.; STROBEL, H.J. Effect of ionophores on ruminal fermentation, **Applied and Environmental Microbiology**, Washington, v.55, n.1, p.1- 6, 1989.

SAMPAIO, C.B.; DETMANN, E.; PAULINO, M.F.; VALADARES FILHO, S.C.; SOUZA, M.A.; LAZZARINI, I.; PAULINO, P.R.; QUEIROZ, A.C. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds, **Tropical Animal Health and Production**, v.42, p.1471-1479, 2010.

SAS, SAS/STAT-User's guide, 9,2, Cary, NC, USA: SAS Inst, Inc, 2010,

SCHELLING, G.T. Monensin mode of action in the rumen, **Journal of Animal Science**, v.58, p.1518-1527, 1984,

SILVA, M.M.C.; RODRIGUES, M.T.; RODRIGUES, C.A.F.; BRANCO, R.H.; LEÃO, M.I.; MAGALHÃES, A.C.M.; MATOS, R.S. Efeito da suplementação de lipídios sobre a digestibilidade e os parâmetros da fermentação ruminal em cabras leiteiras, **Revista Brasileira de Zootecnia**, v.36, p.246-256, 2007.

SIMPSON, M.E. Effects of certain antibiotics in vitro cellulose digestibility and volatile fatty acid production by ruminal microorganisms. **Journal of Animal Science**, v.47, Sup.1, p. 429 (Abstr.), 1980.

UEDA, K.; FERLAY, A.; CHABROT, J.; LOOR, J.J.; CHILLIARD, Y.; DOREAU, M. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage:concentrate ratios, **Journal of Dairy Science**, v.86, p.3999–4007, 2003.

USHIDA, K.; JOUANY, J.P.; DEMEYER, D.I. Effects of presence or absence of rumen protozoa on the efficiency of utilization of concentrate and fibrous feeds. In: **Physiological aspects of Digestion and Metabolism in Ruminants** (Tsuda T, Sasaki Y, Kawashima R, eds) Academic Press, Tokyo, p. 625-654, 1991.

VALENTE, T.N.P.; DETMANN, E.; VALADARES FILHO, S.C.; CUNHA, M.; QUEIRÓZ, A.C.; SAMPAIO, C.B. In situ estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. **Revista Brasileira de Zootecnia**, v.40, p.666-675, 2011.

VALINOTE, A.C.; NOGUEIRA FILHO, J.C.M.; LEME, P.R.; SILVA, S.; CUNHA, J.A. Fontes de lipídio e monensina sódica na fermentação, cinética e degradabilidade ruminal de bovinos. **Pesquisa Agropecuária Brasileira**, Brasília, v.41, n.1, p.117-124, 2006.

VAN NEVEL, C.; DEMEYER, D.I. Lipolysis and biohydrogenation of soybean oil in the rumen in vitro: inhibition by antimicrobials. **Journal of Dairy Science**, v.78, p.2797-2806, 1995.

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, n.10, p.3583–3597, 1991.

WALDO, D.R. Effect of forage quality on intake and forage-concentrate interactions. **Journal of Dairy Science**, v.69, n.4, p.617-631, 1986.

WEDEGAERTNER, T.C.; JOHNSON, D.E. Monensin effects on digestibility, methanogenesis and heat increment of a cracked corn-silage diet fed to steers, **Journal of Animal Science**, v.57, p.168–177, 1983.

WEISS, W.P.; PINOS-RODRIGUEZ, J. M. Production responses of dairy cows when fed supplemental fat in low- and highforage diets. **Journal of Dairy Science**, v.92, p.6144-6155, 2009.

ZEOULA, L.M.; DO PRADO, O.P.P.; GERON, L.J.V.; BELEZE, J.R.F.; AGUIAR, S.C.; MAEDA, E.M. Total digestibility and in situ degradability of bulky diets with the inclusion of ionophores or probiotics for cattle and buffaloes. **Semina-Ciencias Agrarias**, v.35, n.4, p.2063–2076, 2014.

CHAPTER V.
POTENTIAL OF BIOGAS PRODUCTION FROM WASTE OF
NELLORE CATTLE FED DIETS SUPPLEMENTED WITH
VEGETABLE OILS AND MONENSIN

5. POTENTIAL OF BIOGAS PRODUCTION FROM WASTE FROM NELLORE CATTLE FED DIETS SUPPLEMENTED WITH VEGETABLE OILS AND MONENSIN

Abstract: The objective with the present study was to evaluate the effect of combination between monensin and vegetable oils inclusion in diets on carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) productions during anaerobic biodigestion process. For the experiment, batch type experimental biodigesters with a load of 2 kg of substrate (water, inoculum, feces and urine) were allocated in a climatic chamber (30 to 35 °C). They were distributed in a completely randomized design, in a 2 x 4 factorial arrangement of treatments, represented by animal wastes that received four diets that differed from the lipid source used (control, canola, soybean and sunflower - inclusion of 3.5% DM in diets and the addition or not of monensin sodium (300 mg / d - about 32 mg / kg DM), resulting in 8 treatments with 4 replicates, totaling 32 experimental units. The biodigester substrate was prepared to achieve a total solid content (TS) of 5.4% and an initial mean pH of 6.71 ± 0.17 during 145 days of experimental period. Samples from influent and effluent (biofertilizer) were collected for determination of the efficiency of removal of TS and volatile solids (VS), and neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP), for efficiency determination of the anaerobic biodigestion process. Polyunsaturated fatty acids source (PUFA) present in treatments sunflower (SUN) and soybean (SOY) increased the efficiency of removal of TS in 31.7% and 28.8%, respectively. Treatments canola (CAN), SUN and SOY increased the removal efficiency of VS in 5.20, 24.1 and 20.0%, whereas, NDF was increased by 10.5, 24.0 and 27.0%, respectively, compared to control (CONT) treatment. Productions of biogas, CH₄ and CO₂ increased by CAN treatment (21.8, 19.6 and 29.5%, respectively) compared to CONT treatment. The potential production of CH₄/feces (L/g) and CO₂/feces (L/g) were greater in treatment CAN in 18.5 and 33.3%, respectively, when compared to control. Monensin reduced the potential production of CO₂/VS_{ad} (L/g) in animal waste (feces and urine) by 11.0%. Source of MUFA such as canola stimulates greater potential production of biogas and greenhouse gases from animal waste. However, sources of PUFA (soybean and sunflower) are more efficient in enhancing the efficiency of nutrient removal of the biofertilizer obtained from animal waste. The present experiment suggests that lipid sources that have been described to decrease enteric CH₄ emissions result in increased biogas production and CH₄ production in biodigestion process. In the present study, there was no interaction between monensin and vegetable oils inclusion in diets, for any of the

variables evaluated, which show that there are no synergic or additive effect for potential biogas production and its composition.

Key words: Biodigestion. Biodigester. Cattle waste. Methane. Carbon dioxide

5.1 INTRODUCTION

The atmospheric concentration of greenhouse gases has been increasing in recent decades and has a significant influence on the climate of Earth, causing global warming. The most important greenhouse gases (GHG) include carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O) and fluorinated gases (USEPA, 2007). Global cattle production is recognized as a sector that contributes to GHGs emission, particularly carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), with values ranging from 10-12% (SMITH et al., 2007), 14.5% (GERBER et al., 2013) and 18% (STEINFELD et al., 2006). The United Nations of Food and Agriculture Organization (FAO) mentions that the livestock sector would account for 9% of global CO₂ emissions (YAMULKI, 2005), 35-40% of CH₄ emissions and 65% of N₂O emissions (STEINFELD et al., 2006). Ruminant production systems, particularly livestock, are associated with environmental impact problems (climate change, land degradation), where CH₄ emission from enteric fermentation has the greatest contribution of GHGs (STEINFELD et al., 2006; GERBER et al., 2013). Cattle contribute with CH₄ gas emissions through the enteric fermentation process and by faecal excretion (VEYSSET et al., 2010). According to this, from CH₄ produced by enteric fermentation in the rumen, 95% is excreted by eructation (DINI et al., 2012), and of that produced in the posterior digestive tract, 89% is excreted through respiration and only 11% by the anus (MURRAY et al., 1976).

Biogas production from cattle feces is presented as a clean, ecologically correct and renewable alternative of the energy generated from animal waste when microorganisms degrade organic materials in an oxygen-free environment, reducing the polluting potential due to the reduction of greenhouse in the atmosphere (RODRIGUES et al., 2014b). On the other hand, the residue resulting from the process (effluent) can be used as organic fertilizer, as its composition is rich in elements required by the plants (GINTING, 2007). Bovine feces are an excellent substrate for biogas production when co-digested with other types of waste such as organic industrial waste, household waste and sewage sludge (IEA, 2005), because the feces are a carrier substrate characterized by the high content of water,

which acts as a solvent for dry materials, due to its high buffering capacity, which regulates the ideal pH in the reactor and by the high level of nutrients, for optimal growth of bacteria (ANGELIDAKI; ELLEGAARD, 2003). Anaerobic digestion of bovine feces contributes with approximately 63% of the biogas produced in this process.

It has been suggested that the strategies used to reduce CH₄ from enteric fermentation of ruminants can be followed by an increase in CH₄ production from feces and a subsequent increase in biogas production. Külling et al. (2002) and Hindrichsen et al. (2005) showed an increase in CH₄ production from feces due to high concentrate supplementation in the diet of dairy cows, while enteric CH₄ emission was reduced. On the other hand, Moller et al. (2004) carried out the anaerobic biodigestion of bovine waste (feces and urine) and observed a linear increase in potential biogas production as the amount of forage in the diet was reduced. The authors showed an increase of 50 L of biogas per kg of added volatile solids (VS) when the proportion of dietary forage fell from 100 to 77%. When compared the effects of waste from cattle fed different forage:concentrate ratio diets (60:40 vs. 40:60, respectively), Orrico Júnior et al. (2012) observed that the diet with higher concentrate content led to a higher efficiency in gas production, with a potential biogas production of 420 L per kg of added VS. This shows that several studies have been carried out to evaluate nutritional strategies on potential biogas production from cattle waste. However, most of them have been researching the addition of high levels of concentrate in animals' diets. According to this, few studies have evaluated the potential for biogas production and the composition of the same from waste of animals fed diets supplemented with monensin (WILDENAUER et al., 1984) or different sources of fatty acids (KÜLLING et al., 2002). Cirne et al. (2007); Gonçalves et al. (2012) and Sunada et al. (2018) have evaluated the potential of lipids in the form of oil residues as a substrate directly mixed with bovine waste to supply biodigesters. According to Hunter Long et al. (2012), the use of lipid residues has been identified as a strategy to expand biogas yield by 30% or more. This behavior can be verified in a study conducted by Orrico Júnior and Orrico (2015) which evaluated bovine waste and addition of 15% of crude glycerin with high lipid content. These authors reported yields of up to 422.09 L of biogas per kg of added VS, with a 53% increase in yield relative to non-use of glycerin. However, all of these researches have focused on evaluating the ideal oil inclusion dose to be used in the co-digestion process with bovine waste, because despite the beneficial effects, there are factors that limit the use of this substrate, such as the clogging of the

system, as well as, the toxic action of long chain fatty acids against the microbiota that acts in the biodigestion process (MATA-ALVAREZ et al., 2014).

From the previous exposed, little research has been done on vegetable oils inclusion in bovine diet and its effect on potential biogas production from animal waste collected directly from the animal without having to directly add the oils to the biodigester. Likewise, biogas production rate, biogas composition (CH_4 , CO_2 and N_2O) expressed in L/d or in L/g of added ou reduced VS, as well as, efficiency of total solids (TS), VS or nutrients (CP, NDF and ADF) removal (%), obtained from waste of cattle fed diets supplemented with vegetable oils in combination with monensin, are not yet characterized. Therefore, it is of great importance to identify if the combination of these factors generates a synergic or additive type effect on the modulation of biogas production and efficiency of nutrient removal, or if, on the contrary, the combination of these factors results in an antagonistic type effect.

It is expected that the supplementation of cattle diets with monensin, vegetable oils sources or their combination will result in increased reduction of solids and fibrous constituents, as well as, CH_4 emissions from bovine waste, as a result of potentialization of biodigestion anaerobic process and decreased N_2O formation in the substrate.

The objective of the present work was to evaluate the effect of the combination of monensin and vegetable oils inclusion in cattle diets on CO_2 , CH_4 and N_2O production during anaerobic biodigestion process as an alternative for bovine waste (biofertilizer) treatment and potential biogas production.

5.2 MATERIALS AND METHODS

The present study was conducted in the Laboratory of Ruminant Nutrition of the College of Veterinary Medicine and Animal Science, University of Sao Paulo (USP), Pirassununga, Brazil. The experiment was conducted according the guidelines established by the Ethics Committee in the Use of Animals of the College of Veterinary Medicine and Animal Science, under the protocol N° 9236160216, in respect of ethical principles of animal experimentation for care and use of animals.

5.2.1 Animal feeding and feces and urine sampling

For the biogas emission potential determination, methane gas (CH₄), carbon dioxide (CO₂) and nitrous oxide (N₂O), as well as, the evaluation of efficiency of nutrient removal (%) of the biofertilizer, feces and urine were collected from eight Nellore cows (494 ± 105 kg of mean body weight) ruminally cannulated not pregnant and non-lactating. Animals were randomly distributed to one of eight experimental diets, isoenergetic (1.2 Mcal of NE_g/kg of DM) and isoproteic (14% of CP), that differ by the lipid source used and the addition or not of sodium monensin (Rumensin® 200, Elanco Animal Health, Brasil). The treatments were: Control (CONT), diet with inclusion of vegetable oils and monensin, canola (CAN), soybean (SOY) and sunflower (SUN). The inclusion of lipid sources in diets was 3.5% DM as oil. Besides the vegetable oils, each cow in a square daily received 300 mg (approximately 32 mg / kg DM) of sodium monensin (Rumensin® 200, Elanco Saúde Animal, Brasil) during the whole experimental period, delivered twice a day (150 mg at 08h00 and 150 mg at 16h00), mixed with the diet.

Diets were offered twice a day, at 08h00 and 16h00 as total mixed ration (60:40, forage:concentrate ratio), for *ad libitum* intake (5 - 10% of leftovers). In all diets the forage source used was corn silage. Forage and concentrate feeds were weekly sampled for DM content determination and diets were adjusted considering their variation in DM content. The amount of forage and concentrate daily offered was adjusted depending on leftovers from the day before. Diets were formulated to attend nutritional requirements for beef cattle, according NRC (2000). The proportion of ingredients and chemical composition are described at Table 24.

For feeding, feces, and urine sampling, animals were distributed in a 4 x 4 replicated Latin square, in a factorial arrangement of treatments 2 x 4 (regarding addition or not of sodium monensin and lipids source inclusion in diets). The experimental unit was the animal inside each period, totalizing 32 experimental units. Each experimental period had 26 days (19 days for diet adaptation) totalizing 104 days of experiment. Feces sampling was manually collected between days 20 and 24, via rectum, twice a day (08h00 and 16h00), while urine collection was performed on day 26 of each experimental period every 6 hours (at 06h00, 12h00, 18h00 and 24h00), by vulva massage. Urine spot samples of 50 mL were filtered and placed in flasks. All samples were stored at -20 °C in a single vial per animal and per experimental period, composing a single sample of 24 hours, until homogenization and filling of the biodigesters.

Table 24 - Proportion of ingredients and chemical composition of experimental diets expressed as percentage of dry matter (% DM).

Item	Experimental diets ¹			
	CONT	CAN	SOY	SUN
Ingredients, % DM				
Corn silage	60.9	61.9	61.9	61.9
Ground corn grain	22.6	14.0	14.0	14.0
Soybean meal	13.4	17.5	17.5	17.5
Vegetal oil	-	3.5	3.5	3.5
White salt	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.1	0.1	0.1	0.1
Limestone	0.5	0.5	0.5	0.5
Mineral mixture ²	2.0	2.0	2.0	2.0
Chemical composition				
Dry matter, %	54.1	53.8	53.8	53.8
CP, % of DM	14.6	14.9	14.6	14.1
RDP, % of CP ³	66.8	69.4	69.4	69.4
RUP, % of CP ³	33.2	30.6	30.6	30.6
NDF, % of DM	40.6	38.1	41.8	40.3
ADF, % of DM	26.0	24.7	29.4	29.6
NFE, % of DM	34.6	33.3	31.7	30.0
Ash, % of DM	7.1	7.3	7.3	7.3
Ca, % of DM	0.89	1.07	1.07	1.07
P, % of DM	0.30	0.29	0.29	0.29
EE, % of DM	3.1	6.4	6.3	6.6
TDN, % of DM	76.3	78.1	78.0	77.6
NE _m , Mcal/kg of DM ⁴	1.83	1.88	1.88	1.87
NE _g , Mcal/kg of DM ⁴	1.20	1.25	1.24	1.23

¹CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil. Monensin was included in respective diets at 300 mg per animal per day – approximately 32 mg/kg of DM; ²Mineral and vitamin premix, composition per kg of product: 200 g of Ca, 60 g of P, 20 g of S, 20 g of Mg, 70 g of Na, 15 mg of Co, 700 mg of Cu, 700 mg of Fe, 40 mg of I, 1,600 mg of Mn, 19 mg of Se, 2,500 mg of Zn; ³Values estimated by the software *Nutrient Requirements of Beef Cattle: Seventh Revised Edition (2000)*. ⁴ME (Metabolizable energy) = [(TDN kg x 0.04409) x 0.82] x 100 (NRC, 2000); equations to convert ME values into NE_m and NE_g (Mcal/kg DM) have been reported by Garrett (1980) and NRC (2000).

For determination of fatty acids profile of oils included in experimental diets (Table 25), the extraction was performed by methodology described by Folch et al. (1957), by sample homogenization with a solution composed by chloroform and metanol 2:1 in homogenizer Ultra Turrax Marconi®. Lipids were isolated after the addition of NaCl 1.5%

solution. For methylation, methyl esters were formed according methodology described by Kramer et al. (1997). Fatty acids were quantified by gas chromatography (CG-2010 Plus - Shimadzu, auto injector AOC 20i), using capillary column SP-2560 (100 m $\times$ 0.25 mm of diameter with 0.02 mm of thickness, Supelco, Bellefonte, PA). The initial temperature of column was 45°C, with progressive heating until reaching 175°C, remaining for 27 minutes. After, a further increase of 4 °C/min was started up to 215°C, remaining for 35 minutes. Hydrogen (H₂) was used as a carrier gas with flux of 40 cm³/s. The temperature used by the flame ionization detector (FID) was 250°C, H₂ flow of 40 mL/min, air flow of 400 mL/min, make-up of 30 mL/min kPa (N₂) and sampling rate of 40 msec. The total time of each run per sample (stop time) was 86 minutes.

Fatty acids were identified according retention time of methyl esters of samples using the standard solutions 463 Nu-Chek®, vaccenic acid C18:1 trans-11 (V038-1G, Sigma®), C18:2 trans-10 cis-12 (UC-61M 100mg), CLA and C18:2 cis-9, trans-11 (UC-60M 100mg) and tricosanoic acid (Sigma®). Fatty acids were quantified by normalization of the peak area of the methyl esters, using the GS solution software 2.42®. Fatty acids were expressed as percentage of total methyl ester quantified.

5.2.2 Substrate, treatments and experimental design

Thirty-two batch type biodigesters were built to contain a single load of 2 kg of substrate (water, inoculum, feces and urine). The 15 cm and 7.5 cm cylinders were inserted one inside the other, such that the space between the external wall of the inner cylinder and the internal wall of the outer cylinder contained a volume of water (water seal), reaching 60 cm depth. The intermediate diameter cylinder (gasometer) had one of the sealed ends, with only one record remaining to discharge the biogas, and was overlaid on the water seal, to provide anaerobic conditions and store the gas produced, as shown in Figure 11.

In order to supply the biodigesters, each substrate was prepared with 1.496 kg of water, 0.333 kg of inoculum (from wastewater treatment pond), 0.250 kg of urine and 0.921 kg of feces, reaching a total solids (TS) content of 5.4% and an initial average pH of 6.71 ± 0.17 . For a total of 3 kg of prepared substrate, 2 kg were used to fill the biodigesters and 1 kg was sampled for substrate characterization analyzes. After being filled, the biodigesters were conditioned in a climatic chamber with a controlled temperature between 30 and 35°C, where they remained throughout the evaluation period (145 days), ensuring that the anaerobic digestion test was carried out under mesophilic conditions. The ambient

temperature was monitored by means of a digital thermometer (in °C), before biogas sampling produced in each biodigester.

Table 25 - Fatty acids profile of oils used in experimental diets.

Fatty acid ¹	Canola oil	Sunflower oil	Soybean oil
Lauric, C12:0	0.021	0.008	0.001
Myristic, C14:0	0.061	0.065	0.079
Palmitic, C16:0	4.445	6.148	11.09
Palmitoleic, C16:1 trans 9	0.036	0.013	0.008
Palmitoleic, C16:1 cis 9	0.219	0.091	0.080
Heptadecanoic, C17:0	0.080	0.038	0.071
Heptadecenoic, C17:1 cis 10	0.115	0.027	0.042
Stearic, C18:0	2.334	3.870	3.330
Oleic, C18:1 n-9 cis	56.23	37.67	21.45
Vaccenic, C18:1 cis 11	6.294	0.132	2.389
Linoleic, C18:2 n-6 cis	18.69	50.00	53.38
γ-linolenic, C18:3 n-6	0.421	0.010	0.097
Linolenic, C18:3 n-3	8.546	0.242	6.427
Araquidic, C20:0	0.020	-	0.317
Eicosenoic, C20:1 cis 5	0.062	0.007	0.022
Eicosenoic, C20:1 cis 8	0.365	0.004	0.083
Eicosadienoic, C20:2 n-6 cis 11,14	0.045	0.005	0.032
Eicosatrienoic, C20:3 n-6 cis 8, 11, 14	0.363	0.662	0.421
Eicosatrienoic, C20:3 n-3 cis 11, 14, 17	0.043	0.014	0.005
Heneicosanoic, C21:0	0.011	-	0.020
Tricosanoic, C23:0	0.021	0.019	0.038
Eicosapentaenoic, C20:5 n-3 cis (EPA)	0.162	0.188	0.125
Docosatrienoic, C22:3 n-3 cis 13, 16, 19	0.123	0.015	0.015
Docosatetraenoic, C22:4 n-6	0.009	-	-
Docosahexaenoic, C22:6 n-3 cis (DHA)	0.008	-	0.005
PUFAS ²	28.41	51.14	60.51
MUFAS ³	63.32	37.94	24.09
SFAS ⁴	7.011	10.16	14.95
n-3	8.882	0.459	6.577
n-6	19.52	50.67	53.93
n-6/n-3	2.197	110.3	8.199

¹Fatty acids expressed in percentage (%) of the sample; ²PUFA: Polyunsaturated fatty acids; ³MUFA: Monounsaturated fatty acids; ⁴SFA: Saturated fatty acids.

Figure 11. Illustration of an anaerobic biodigester batch type.

Source: Romero, 2019.

The treatments were determined based on the feces collected from the cows that received the different experimental diets, with 8 treatments and 4 replicates. The biodigesters were arranged in a completely randomized design in a 2 x 4 factorial arrangement (referring to the monensin and lipid source factors, respectively), totaling 32 experimental units.

5.2.3 Measurement of production and sampling of biogas

The reading of biogas volume was carried out with the help of a strip of tape fixed in the gasometer, according to the vertical displacement of the gasometer due to the accumulation of gas inside it. The monitoring of the height (cm) of the gasometer was daily performed, and a biogas sample collection was performed when the height was greater than 28 cm and less than or equal to 40 cm. The volume of biogas obtained at the reading was multiplied by the internal cross-sectional area of the gasometer (0.00785 m^2) and corrected for the conditions of 1 atm at 20°C as described by Lucas Junior (1994). For biogas volume correction, an equation resulting from the combination of the laws of Boyle and Gay-Lussac was used, where:

$$\frac{V_0 P_0}{T_0} = \frac{V_1 P_1}{T_1} \quad (1)$$

where:

V_0 = Corrected volume of biogas, m³ or L;

P_0 = Corrected pressure of biogas, 10,322.72 mm of H₂O;

T_0 = Corrected temperature of biogas, 293.15 K;

V_1 = Gas volume at the gasometer;

P_1 = Biogas pressure at the instant of reading, 10,308.11 mm de H₂O;

T_1 = Biogas temperature, in K, at the instant of reading.

Considering the average atmospheric pressure of Pirassununga - SP (Brazil) equal to 10,273.11 mm of water and pressure checked by gasometers of 71 mm of water, the following expression was obtained to correct the volume of biogas:

$$V_0 = \left(\frac{V_1}{T_1} \right) \times 293.7703 \quad (2)$$

Biogas sampling was performed after the reading of the gasometer displacement (volume of biogas). Biogas was collected from the top of the gasometer with the help of a 3-way valve and a 60-mL syringe. Prior to the collection, in order to mix the gases inside the gasometer and to avoid the collection of some less dense gases, which tend to be in the upper part of the gasometer, the gases were homogenized by the syringe. Afterwards, a 3-way valve was fitted to collection flasks (50 mL flask of transparent glass, Frascolex, São Paulo, Brazil). By the syringe, biogas was injected through one of valve way. From another way of the valve the gas was expelled, ensuring the ejection of all air present in the flask (flushing of the flask). This procedure was performed 3 times in each flask. After washing, 50 mL of biogas sample was collected from the gasometer and injected into the glass bottles to evaluate its composition.

After the production reading and the biogas samples were withdrawn, the gasometers were restarted using the biogas discharge register, thus allowing a new accumulation of gas throughout the period.

5.2.4 Biogas composition

The concentrations of CH₄, CO₂ and N₂O were determined by gas chromatography in a temperature-controlled environment (25°C), according to Kaminski et al. (2003). The gas chromatograph (Trace 1310, Thermo Fisher Scientific®, Rodano, Milan, Italy) was equipped with an injection system (split/splitless), two stainless steel columns 2 m long and 2 mm internal diameter, packed with stationary phase Porapak Q 80/100 (Restek), both coupled to a stainless steel pre-column 1 m long and 2 mm internal diameter packed with Hayseep Q 80/100 phase (Restek). One of the columns had methanizer followed by the flame ionization detector (FID), responsible for the measurement of CO₂ and CH₄. The other column had the electron capture detector (ECD), responsible for the quantification of N₂O. The conditions of analysis for the FID were: temperature of 250°C, hydrogen carrier gas (70 KPa), hydrogen flame production (20 mL/min), synthetic air (300 mL/min) and make-up (15 mL/min). For the ECD detector, the conditions were: temperature 350°C, nitrogen carrier gas (65 KPa), make-up (15 mL/min), current reference of 0.5 nA, pulse amplitude 50 V and pulse width 1.0 µs.

The calibration of the gas chromatograph was performed using a mixed gas standard containing 3.11% of CH₄, 3.11% of CO₂ and 0.49% of N₂O diluted in atmospheric air using two standard reference materials containing 50% CH₄ and 50% CO₂ (mol/mol) and another 10% N₂O in balance with He (mol/mol). Helium with flow rate of 30 mL/min was used as the carrier gas.

Biogas samples were diluted in glass vials with previously known volume, 16.78 times in atmospheric air. Manually using a syringe, 6 mL of the diluted sample were injected into the chromatograph injector (split/splitless), where 4 mL were used to wash the injection system and 2 mL were used for analysis, 1 mL for the FID system and 1 mL for the ECD. The number of replicates per sample was the number required to make the difference between readings less than 5%.

5.2.5 Measurement of methane, carbon dioxide and nitrous oxide gases production

The measurement of potential production of CH₄, CO₂ and N₂O was carried out from the biogas production (L) and concentration (%) of the different gases in the sampled biogas. The volume of CH₄, CO₂ and N₂O produced (m³) was calculated by multiplying

the biogas production and composition during the anaerobic biodigestion process, according to the equation:

$$\text{Vol} = \frac{\text{Vol Biogás} \times \% \text{ gás}}{100} \quad (3)$$

where:

Vol = Volume of measured gas (m³)

Vol_{BIOGAS} = Volume of produced biogas (m³)

% gas = Concentration of gas in biogas (%).

The gas production in relation to volatile solids (VS) added and reduced was calculated by the division of the total gas production (CH₄, CO₂ in L and N₂O in mL) and the amount of VS added or reduced in g.

5.2.6 Kinetics of biogas production

The gas production curve was estimated from the production of CH₄, CO₂ (both in L) and N₂O (in mL), using the Gompertz model analyzed by the Gauss-Newton method of SAS. This model assumes that the rate of gas production is proportional to the microbial activity, but the proportionality decreases with the incubation time, which can be interpreted as the loss of efficiency in fermentation rate (LAVRENCIC et al., 1997). The mathematical description of gas production curves allows the data analysis, the comparison of the substrates and the performance of the fermentation, according to Gompertz model:

$$y = A \exp [-B \exp (-kt)] \quad (4)$$

where:

y = Gas production (CH₄ and CO₂, L/g and N₂O, mL/g of added VS) at time t (day);

A = Asymptote of the model, indicates the stabilization value of production (CH₄ and CO₂, L/g and N₂O, mL/g of added VS) at time t;

B = Integration constant, paraneter of time scale parameter with no specific biological meaning;

k = Maximum production rate, logarithmic function of production (CH₄ and CO₂, L/g/d and N₂O, mL/g/d of added SV) per unit time;

t = Time at inflection point (day).

5.2.7 Laboratory analysis

To determine the characteristics and quality of the biofertilizer, samples were collected from the substrate that feed the biodigesters (influent) and the resulting residue (biofertilizer). Samples were dried at 55°C for 72 h in a forced-air ventilation oven and ground to a particle size of 1 mm in a Willye-type mill to determine dry matter (DM), performed at 105°C for 4 h (DM - Method 934.01), mineral matter (MM - Method 923.03), and crude protein (CP - Method 920.87) by total N determination using the micro-Kjeldahl technique and multiplying the obtained value by 6.25 as indicated by the AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991).

Total solids contents (TS at 105°C = 100 - humidity) and volatile solids (VS = TS - MM) of the samples were determined with adaptations to the methodology described at APHA (2005). Total solids and volatile solids in g/kg were obtained by multiplying the percentage of total solids and volatile solids by 10, respectively.

The hydrogen potential (pH) was measured by portable pH meter (Hanna Instruments®, HI 8424, Italy).

5.2.8 Nutrients removal

Added (influent) and eliminated (biofertilizer) substrates of each biodigester were weighed and multiplied by their DM content, in percentage, to calculate the DM content in grams (g). The nutrients added and eliminated, expressed in g, were calculated by multiplying the concentration of DM (g) and its respective nutrient concentration (NDF, ADF or CP, %), divided by 100, as indicated in the following equation:

$$\text{Nutrients (g)} = \left(\frac{\text{DM (g)} \times \text{added or eliminated nutrient (\%)}}{100} \right) \quad (5)$$

where:

Nutrient (%) = Concentration of NDF, ADF or CP in added or eliminated substrate.

Nutrient removal (%) were calculated from the content of added and eliminated nutrients, expressed in grams per kilogram of DM, according to the following formula:

$$\text{Nutrient removed (\%)} = \left(\frac{\text{Nutrient added (g)} - \text{Nutrient eliminated (g)}}{\text{Nutrient added (g)}} \right) \times 100 \quad (6)$$

5.2.9 Statistical analyses

Results were analyzed by the Statistical Analysis System (Version 9.4, SAS Inst., Inc., Cary, NC, USA 2015), after normality of residues was verified by the Shapiro-Wilk test (PROC UNIVARIATE) and homogeneity of variances compared by the Hartley test. Data were submitted to analysis of variance, which separated as causes of variation the effect of oil, monensin effect and its interaction. In the presence of oil effect, the comparison of means was done by the adjusted Tukey test. A significance level of 5% was adopted. The following statistical model was used:

$$Y_{ij} = \mu + O_i + M_j + (O_i \times M_j)_{ij} + e_{ij}$$

where:

Y_{ijkl} = Observation related to Oil (i) + Monensin (j) + Oil (i) x Monensin (j) + random error associated to each observation (e_{ijkl});

μ = Average mean associated to the variable;

O_i = Fixed effect of oil i;

M_j = Fixed effect of monensin j;

$(O_i \times M_j)_{ij}$ = Interaction effect between oil (i) and monensin (j);

e_{ijkl} = Experimental random error associated to each observation.

5.3 RESULTS

5.3.1 Biogas production

There was an increase ($P<0.05$) in biogas, methane (CH_4) and carbon dioxide (CO_2) potential production from the waste (feces and urine) of animals that received CAN treatment (21.8%, 19.6% and 29.5%, respectively) compared to CONT treatment (Table 26). Similar effect was observed for the potential production of CH_4 /feces (L/g) and CO_2 /feces (L/g), which was greater in CAN treatment in 18.5% and 33.3%, respectively, in relation to CONT treatment.

The potential production of CO_2 /VS ad (L/g) was reduced ($P=0.0478$) in the waste of animals that received monensin in 11.0%, compared to the waste from animals not supplemented with monensin.

In the absence of monensin supplementation, animals had less time to reach the inflection point (t) for the production of CH_4 ($P=0.0001$), CO_2 ($P=0.0007$) and N_2O ($P=0.0001$) in 22.5%, 21.9% and 23.8%, respectively, when compared to animals from monensin group, as indicated in Figure 12.

There was no interaction effect between monensin and vegetable oils inclusion in diets for any of the evaluated variables, which shows that there was no synergistic or additive effect for biogas potential production and its composition.

5.3.2 Biodigestion and nutrients removal

Animal waste (feces and urine) added to the biodigesters from animals fed CAN, SUN and SOY treatments increased ($P=0.0329$) NDF content (g) in 9.2, 12.5 and 10.7%, respectively. The other variables of added nutrients were not significantly affected ($P>0.05$) by oil or monensin inclusion in diets (Table 27).

For eliminated nutrients (remaining nutrients after anaerobic biodigestion process), no significant effect ($P>0.05$) of oil inclusion in diets was observed; however, the amount of VS (g) and of ADF (g) were increased in 6.8 and 17.6%, respectively, with the addition of monensin compared to no addition of monensin.

Table 26 - Potential production of biogas, methane, carbono dioxide, and nitrous oxide in biodigesters batch type filled with waste (feces and urine) from Nellore cattle fed diets supplemented with vegetable oils and monensin.

Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
Biogas, L	42.74	41.62	37.98 ^b	46.26 ^a	39.89 ^{ab}	44.58 ^{ab}	1.1048	0.5665	0.0227	0.3674
Methane (CH₄)										
CH ₄ , L	27.58	27.15	24.73 ^b	29.59 ^a	26.04 ^{ab}	29.09 ^{ab}	0.6699	0.7206	0.0263	0.5218
CH ₄ , %	64.57	65.76	65.35	64.31	65.50	65.49	0.4068	0.1324	0.6323	0.0984
CH ₄ /feces, L/g	0.030	0.029	0.027 ^b	0.032 ^a	0.028 ^{ab}	0.031 ^{ab}	0.0007	0.7085	0.0259	0.5237
CH ₄ /VS ad, L/g	0.320	0.298	0.288	0.328	0.301	0.319	0.0069	0.1072	0.1660	0.6112
A, L/g	0.317	0.301	0.288	0.328	0.301	0.319	0.0070	0.2614	0.2122	0.7128
k, L/g/d	0.043	0.041	0.040	0.040	0.045	0.043	0.0012	0.3397	0.5103	0.4770
t, d	32.26	41.65	37.03	38.75	35.02	37.03	1.2703	0.0001	0.6256	0.6417
y, L/g	0.117	0.111	0.106	0.120	0.110	0.117	0.0025	0.2614	0.2122	0.7128
CH ₄ /VS red, L/g	1.155	1.079	1.192	1.118	1.017	1.141	0.0612	0.5508	0.7854	0.1731
Carbon dioxide (CO₂)										
CO ₂ , L	10.12	9.556	8.623 ^b	11.16 ^a	9.262 ^{ab}	10.30 ^{ab}	0.3104	0.2934	0.0145	0.3288
CO ₂ , %	23.44	22.39	22.51	23.41	23.09	22.64	0.3248	0.1152	0.7399	0.2636
CO ₂ /feces, L/g	0.011	0.010	0.009 ^b	0.012 ^a	0.010 ^{ab}	0.011 ^{ab}	0.0003	0.3001	0.0149	0.3415
CO ₂ /VS ad, L/g	0.118	0.105	0.101	0.124	0.107	0.113	0.0034	0.0478	0.0829	0.4647
A, L/g	0.112	0.093	0.096	0.108	0.094	0.112	0.0048	0.1079	0.5588	0.6676
k, L/g/d	0.041	0.046	0.035	0.045	0.050	0.044	0.0022	0.3470	0.2314	0.3530
t, d	31.55	40.41	35.32	38.55	33.03	37.01	1.3610	0.0007	0.3933	0.7433
y, L/g	0.041	0.034	0.035	0.040	0.034	0.041	0.0017	0.1079	0.5588	0.6676
CO ₂ /VS red, L/g	0.424	0.378	0.421	0.421	0.358	0.404	0.0243	0.3404	0.7551	0.1177
Nitrous oxide (N₂O)										
N ₂ O, mL	10.08	10.54	12.30	7.835	9.221	11.87	0.9177	0.8091	0.2947	0.5243
N ₂ O, %	0.024	0.029	0.033	0.020	0.024	0.027	0.0023	0.3371	0.2957	0.6978
N ₂ O /feces, mL/g	0.010	0.011	0.013	0.0085	0.010	0.012	0.0009	0.8091	0.2947	0.5243
N ₂ O /VS ad, mL/g	0.118	0.116	0.147	0.087	0.103	0.131	0.0108	0.9386	0.2625	0.6101
A, mL/g	0.042	0.054	0.035	0.027	0.039	0.091	0.0136	0.6936	0.4120	0.7205
k, mL/g/d	0.044	0.042	0.040	0.043	0.045	0.044	0.0014	0.5915	0.7300	0.4783
t, d	31.88	41.85	37.03	39.25	35.22	35.96	1.3672	0.0001	0.5548	0.4817
y, mL/g	0.017	0.020	0.028	0.010	0.014	0.033	0.0055	0.7728	0.4873	0.9786
N ₂ O /VS red, mL/g	0.356	0.409	0.488	0.349	0.332	0.361	0.0341	0.4466	0.4454	0.3485

¹CH₄/feces, L/g: methane production in relation to weight (fresh matter) of feces added; CH₄/VS ad, L/g: methane production in relation to volatile solids added; CH₄/SV red, L/g: methane production in relation to volatile solids reduced; CO₂/feces, L/g: carbon dioxide production in relation to weight (fresh matter) of feces added; CO₂/VS ad, L/g: carbon dioxide production in relation to volatile solids added; CO₂/SV red, L/g: carbon dioxide production in relation to volatile solids reduced; N₂O/feces, mL/g: nitrous oxide production in relation to weight (fresh matter) of feces added; N₂O/VS ad, mL/g: nitrous oxide production in relation to volatile solids added; N₂O/SVred, mL/g: nitrous oxide production in relation to volatile solids added; A: Assintotic production; k: constant of production (L/g.d); t: time at inflexion point (day); y: production at inflexion point (L/g); ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statically differ by Tukey test ($P < 0.05$).

Figure 12 - Curves for cumulative production of CH_4 (a. and b.), CO_2 (c. and d.), N_2O (e. and f.) adjusted by the Gompertz model in batch anaerobic digesters filled with animal waste (feces and urine) from Nellore cattle fed diets supplemented or not with monensin and vegetable oils.

Source: Romero, 2019.

Treatments SUN and SOY increased efficiency of TS removal (%) by 31.8 and 28.8%, respectively (Table 27). For VS (%), its efficiency was increased by CAN, SUN and SOY in 5.2, 24.1 and 20.0%, whereas for NDF (%) the increase was of 10.5, 24.0 and 27.0%, respectively, in relation to control treatment. For ADF and CP, there was no oil effect ($P>0.05$). However, monensin effect ($P=0.0179$) was observed for efficiency of ADF removal (%), which was reduced in 31.6% with the addition of monensin.

No interaction MxO was observed for any evaluated variable.

Table 27 - Biodigestion and efficiency of nutrients removal (%) from biodigesters batch type filled with waste (feces and urine) from Nellore cattle fed diets supplemented with vegetable oils and monensin.

Vegetable Oils and Monensin.										
Variables ¹	Monensin		Oil ²				SEM ³	P-value ⁴		
	Without	With	CONT	CAN	SUN	SOY		M	O	MxO
Added nutrients										
pH	6.66	6.75	6.58	6.69	6.79	6.74	0.0292	0.1012	0.0505	0.5148
TS, g	106.0	108.6	104.3	108.6	107.2	109.1	0.9351	0.1801	0.2654	0.7366
VS, g	88.19	91.18	86.85	90.62	90.16	91.12	0.8855	0.0997	0.3087	0.7495
CP, g	30.51	28.88	29.46	28.47	30.29	30.54	0.6272	0.2262	0.6788	0.7098
NDF, g	54.60	54.26	49.88 ^b	54.97 ^a	56.99 ^a	55.88 ^a	0.8977	0.8419	0.0329	0.9283
ADF, g	39.36	40.57	36.88	40.98	40.33	41.67	0.6992	0.3676	0.0772	0.6048
Eliminated nutrients										
pH	7.41	7.40	7.42	7.35	7.40	7.43	0.0204	0.8114	0.5949	0.8772
TS, g	85.76	89.46	88.58	90.38	85.29	86.20	1.0838	0.0763	0.2886	0.2017
VS, g	62.92	67.48	65.96	67.83	63.05	63.95	1.0035	0.0166	0.2549	0.2252
CP, g	14.09	14.26	14.05	15.16	13.43	14.04	0.2785	0.7507	0.1658	0.2487
NDF, g	28.07	32.12	31.04	31.91	27.58	29.83	1.0039	0.0543	0.4662	0.9377
ADF, g	22.97	27.87	26.20	27.29	22.49	25.72	1.1351	0.0395	0.4850	0.9647
Efficiency of nutrient removal										
TS, %	19.71	17.51	14.90 ^b	16.77 ^{ab}	21.84 ^a	20.93 ^a	1.0826	0.2615	0.0497	0.1659
VS, %	29.23	25.89	23.86 ^c	25.17 ^b	31.43 ^a	29.77 ^{ab}	1.1568	0.1014	0.0341	0.1353
CP, %	53.11	50.18	51.92	46.56	55.22	52.86	1.3399	0.2347	0.1007	0.1359
NDF, %	48.03	41.74	37.38 ^b	41.76 ^a	49.20 ^a	51.19 ^a	1.9079	0.0848	0.0360	0.9707
ADF, %	44.28	30.29	34.53	32.75	44.17	37.71	2.8984	0.0179	0.4749	0.6406

¹TS: Total solids; VS: volatile solids; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ²CONT: Control; CAN: Canola oil; SUN: Sunflower oil; SOY: Soybean oil; ³SEM: Standard error of the mean; ⁴M: Monensin effect; O: Oil effect; MxO: Interaction between monensin and oil; ^{ab}Different letters in the same line statistically differ by Tukey test ($P<0.05$).

5.4 DISCUSSION

In the present study, cattle diets affected the composition of the substrate used to fill the biodigesters, resulting in higher concentration of NDF in the influents from animals

fed lipid sources. The addition of vegetable oils in diets may have resulted in lower fiber digestibility which increased NDF fraction. Similar effect was also observed by Külling et al. (2002).

For total (TS) and volatile (VS) solids content, effluent values (biofertilizer) were lower than influent (substrate) in all evaluated treatments. This behavior indicates efficiency of anaerobic biodigestion through organic matter degradation by bacteria and, consequently, increased biogas production (ITODO; AWULU, 1999; ORRICO JR. et al., 2010).

Higher total solids removal efficiency observed in PUFA treatments is associated to the fact that a large part of them are volatile solids part of raw material for biogas production, which indicates that, if biogas production was greater, the efficiency of removal of this component and volatile solids will be too (OSORIO et al., 2007). This result was directly related to increased volatile removal efficiency with lipid sources addition (MUFA and PUFA).

Total solids of cattle waste vary according to the feed and the amount of water consumed by the animals. According to Horn et al. (1994), the volume of water is the most variable component of cows' waste. From the moment of biodigesters filling, it is important to consider the contents of total and volatile solids, as these are the precursors of CH₄ and CO₂ in anaerobic biodigestion process, and the dilution of the substrate can prevent the inhibition of hydrolysis by free ammonia accumulation and methanogenesis by short chain fatty acids accumulation (VEDRENNE et al., 2008). Volatile solids are considered the main parameter, as they are associated to the biodegradable fraction of the effluent with potential biogas production. The reduction in VS is usually used to evaluate the performance of anaerobic biodigestion process and microbial metabolic activity (KUNZ et al., 2014). Orrico et al. (2010) report that greater reductions in VS can be associated to fresh materials used for biodigester fill and the maintenance of reactors at a constant temperature of 31 °C, as well as, a long retention period (up to 100 days).

Increased efficiency of NDF removal (%) by MUFA and PUFA is directly related to increased biogas and its components production. While, the absence of a significant effect on biogas production when monensin is supplemented may be due to the low biodegradability of fibrous materials, which may reach 40-50% of total solids in cattle feces (BOE; ANGELIDAKI, 2009). This fact is related to reduction in the efficiency of ADF removal when monensin is used. The degradation of solids is associated with ADF

levels in the substrate, and the greater amount of this fraction compromises the degradation of the substrates.

Higher concentrate proportion in cattle diets, fed as TMR diets (ORRICO JR. et al., 2012), or with lipids inclusion may favor the degradation of the substrates in the biodigester, which leads to decreased of fibrous constituents (NDF and ADF), which in smaller quantities favor marked reductions of TS and VS, and, consequently, generates greater biogas potential production. This effect was observed in the present experiment.

Sunada (2018) evaluated the anaerobic co-digestion of animal waste at different waste cooking oil concentrations and verified that the inclusion of cooking oil directly on the substrate of the biodigester provided carbon sources more readily degradable than the dietary fiber present in the animals' diets.

Amaral et al. (2004) observed for biodigesters operated with 30 days of hydraulic retention time (HRT), VS reductions ranging from 29 to 33%. These results were very close to those found by Orrico et al. (2010), where VS reductions were 28.3 and 32.4% for diets with 60 and 40% forage, respectively. In the present study, values between 23 and 31% were observed, close to those mentioned in other studies. Orrico et al. (2010) describe that increasing the proportion of concentrate in animals' diet favors the degradation of substrates and positively impact the efficiency of biodigestion process.

According to this, reductions of TS, VS, NDF, ADF and cellulose of bovine waste from cattle fed 60% concentrate diet were higher to those obtained from cattle waste of animals fed 40% concentrate diet. On the other hand, when studying the co-digestion of feces from dairy cattle with oil directly poured into the substrate of batch biodigesters, Orrico et al. (2016) observed reductions of 47.5% in TS and of 51.3% in VS.

Regarding pH verified in both the substrates and the biofertilizers of the biodigesters, it remained close to neutrality (pH 6.5 to 7.4), practically stable and at levels considered optimal for stabilization and anaerobic biodigestion due to the production of alkaline substances. For the great majority of bacteria, the optimum pH should be between 6.5 and 7.5 (CAMPOS et al., 2006).

Observing the values of the influent and the effluent, for all collection points, effluents had higher values of alkalinity than the influents, indicating that the anaerobic biodigestion process is responsible for alkalinizing substances production, which neutralize the acids produced, raising the resistance to pH drop and maintenance of appropriate levels for better system performance.

Generation of biogas from animal waste is dependent on temperature, pH, alkalinity and management adopted in confined animals' production system, as well as, the characteristic of the residue, which is the substrate for microorganisms growth in the biodigester. The difference in biogas generation capacity is associated to several factors, such as the diet of the animals and the digestive processes, which result in residues with different characteristics and different biogas potential production (KUNZ et al., 2014).

The increase in potential production of biogas, CH₄ and CO₂ gases, when lipid sources rich in PUFA are added in diets, is associated to the fact that, lipids are efficient substrates in improving anaerobic digestion and co-digestion due to higher yield of CH₄ when compared to proteins or carbohydrates. In this context, lipid-rich residues can be considered as a major potential source of renewable energy generation (HANSEN et al., 1999). This indicates that the greater the proportion of methane in biogas, the greater its energy yield for use as a fuel (SOUSSANA et al., 2010).

The potential production of CH₄/feces (L/g) and CO₂/feces (L/g) showed the same behavior as these gases production, expressed in L, increased from MUFA lipid source, such as canola. This result is associated to feces production with higher concentration of nutrients. Lipids are attractive for biogas production as they have a high CH₄ yield potential (ALVES et al., 2009). This is because the addition of a more easily degradable material provides more carbon for microbial metabolism, which consequently results in higher yield of biogas (ORRICO JR; ORRICO, 2015).

The increase in potential biogas and CH₄ production is explained by lipid metabolic behavior in anaerobic environments such as the biodigester, where the lipids are first hydrolyzed in glycerol and free long chain fatty acids (LCFA). This process is catalyzed by extracellular lipases that are secreted by acidogenic bacteria. Additional conversion of hydrolysis products occurs in bacterial cells. Glycerol is converted to acetate by acidogenesis, while LCFAs are converted to acetate and hydrogen via the β -oxidation pathway (synthetic acetogenesis) (WENG; JERIS, 1976). This process depends on the ability of hydrogenotrophic methanogens to use the hydrogen produced during fatty acids oxidation. Alves et al. (2001) observed that after continuously addition of oleic acid (C18:1, MUFA source) in anaerobic reactors, there was increased efficiency of CH₄ production in the substrate. This was verified in batch assays at 37°C and, without addition of any carbon source, showing that the anaerobic consortium remained active under such conditions. It has also been demonstrated that LCFAs, since associated with sludge or sewage residue as inoculum and not with a bulky material, can be converted more

efficiently into CH₄ (PEREIRA et al., 2004, 2005). This mechanism justifies the increase of CH₄ potential production in the present experiment, from waste from animals fed with lipid sources.

The reduction of CO₂/VS_{ad} (L/g) potential production in animal waste from cattle supplemented with monensin was related to decreased ADF removal efficiency, which did not show a significant effect on biogas potential production.

The best variable to reflect the potential of a given biomass and therefore the most suitable for use in biodigester projects is the one that expresses the biogas production per kg of TS added in the biodigesters, because it eliminates the interference of water content present in biomass. The production in terms of volatile solids added refers to the true gas yield (IPCC, 2006). The production of gases in relation to volatile solids added were smaller than the reduced volatile solids. Franco et al. (2007) indicated that the true yield is always less than the theoretical yield because a fraction of the substrate is used to synthesize bacterial mass.

Regarding the potential of gas production in terms of reduced volatile solids, this corresponds to the theoretical potential of gas yield (IPCC, 2006), indicating complete degradation of the whole organic fraction of feces.

The inflection point is characterized by higher speed of biogas production at a given moment, followed by a decrease in production (QUEIROZ, 2001). In the present experiment, the inflection point had effect of monensin. In monensin treatment, the inflection point had close values at 41 days and in no monensin addition treatment, the inflection point had close values at 31 days, for the three gases evaluated.

The increased removal of solids and fibrous constituents from vegetable oils resulted in increased biogas potential production, as well as, CH₄ and CO₂ emissions, which in turn, did not result in increased N₂O formation in the substrate. This was an expected effect, since N₂O is produced in denitrifying environments and the conditions in the biodigestion process were highly reductive. So, under these conditions, N₂O is reduced to the N₂ gas and ends up not persisting in this process (ELGOOD et al., 2010). Nitrous oxide is derived from the degradation of nitrogen compounds such as urea, ammonia and proteins. In this case, the substrate of the biodigesters consisted of lipid sources, which explains why N₂O did not increase during the biodigestion process.

5.5 CONCLUSION

A source of monounsaturated fatty acid, such as canola oil, increases the potential production of biogas and greenhouse gases, such as CH₄ and CO₂, from cattle waste (feces and urine). However, sources of polyunsaturated fatty acids, such as soybean and sunflower, are more efficient in increasing nutrient removal efficiency of the biofertilizer obtained from bovine waste.

The results indicate that there is no interaction effect (MxO) between monensin and vegetable oils inclusion in diets for any of the evaluated variables, which shows that there is no synergistic or additive effect on biogas composition and potential production. However, diets affected the substrate used and at the same time, this was reflected in the influence of biogas production and gas produced.

The present experiment suggests that lipid sources that have been described to decrease enteric CH₄ emissions result in increased biogas production and CH₄ production in biodigestion process.

REFERENCES

- ALVES, D.Z.; MOTA VIEIRA, J.A.; ÁLVARES PEREIRA, R.M.; PEREIRA, M.A.; NOVAIS, J.M.; MOTA, M. Effects of lipids and oleic acid on biomass development in anaerobic in fixed bed reactors. Part I: Biofilm growth and activity. **Water Res.** v.35, n.1, p.255– 263, 2001.
- ALVES, M.M.; PEREIRA, M.A.; SOUSA, D.Z.; CAVALEIRO, A.J.; PICAVET, M.; SMIDT, H.; STAMS, A.J.M.; Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA) – minireview (early view). **Microbial Biotechnology**, v.2, n5, p.538–550, 2009.
- AMARAL, C.C.; AMARAL, L.A.; JÚNIOR, J.L.; NASCIMENTO, A.A.; FERREIRA, D.S.; MACHADO, M.R.F. Biodigestão anaeróbia de dejetos de bovinos leiteiros submetidos a diferentes tempos de retenção hidráulica, **Revista Ciência Rural**, v.34, n.6, p.1897-1902, 2004.
- ANGELIDAKI, I.; ELLEGAARD, L. Codigestion of manure and organic wastes in centralized biogas plants: status and future trends. **Apply Biochemistry Biotechnology**, v.109, n.1-3, p.95-105, 2003.
- AOAC, Association of Official Analytical Chemists, **Official methods of analyses of AOAC International, 18 th**, Current Through Revision, Gaithersburg MD, USA, 2005.
- APHA. Standard methods for the examination of water and wastewater. **American Public Health Association**, Washington. 2005. ISBN 978-0-87553-047-5
- BOE, K.; ANGELIDAKI, I. Serial CSTR digester configuration for improving biogas production from manure. **Water Res.**, v.43, n.1, p.166-172, 2009.
- CAMPOS, C.M.M.; CARMO, F.R.; BOTELHO, C.G.; COSTA, C.C. Development and operation of the up flow anaerobic sludge blanked reator treating liquid effluent from swine manure in laboratory scale. **Revista Ciências e Agroecologia**, v.30, p.140-147, 2006.
- CIRNE, D.G.; PALOUMET, X.; BJORNSSON, L.; ALVES, M.M.; MATTIASSON, B. Anaerobic digestion of lipid-rich waste – effects of lipid concentration. **Renewable Energy**, v.32, n.6, p.965–975, 2007.
- DINI, Y.; GERE, J.; BRIANO, C.; MANETTI, M.; JULIARENA, P.; PICASSO, V. 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, 2012.
- ELGOOD, Z.; ROBERTSON, W.D.; SCHIFF, S.L.; ELGOOD, R. Nitrate removal and greenhouse gas production in a stream-bed denitrifying bioreactor, **Ecological Engineering**, v.36, n.11., p.1575-1580, 2010.
- FOLCH, J.; LEES, M.; SLOANE-STANLEY, G.H. A simple method for the isolation and purification of total lipides from animal tissues. **The Journal of Biological Chemistry**, Baltimore, v. 226, p.497-509, 1957.

FRANCO, A.; MOSQUERA-CORRAL, A., CAMPOS, J.L. Roca Learning to operate Anaerobic bioreactors. **Applied Microbiology**, Spain, 2007.

GARRETT, W.N. Energy utilization by growing cattle as determined in 72 comparative slaughter experiments. **Energy Metab. Proc. Symp.** v.26, p.3–7, 1980.

GINTING, N. Penuntun Praktikum Teknologi Pengolahan Limbah Peternakan. Departemen Peternakan, Fakultas Pertanian, Universitas Sumatera Utara. 2007.

GONÇALVES, M.F.; MARTINS, J.M.S.; OLIVEIRA, M.V.; CARVALHO, C.C.M.; ANTUNES, M. M.; FERREIRA, I.C.; et, al, Ionóforos na alimentação de bovinos, **Vet. Not, Uberlândia**, v.18, n.2, p.131-146, 2012.

HANSEN, K.H.; AHRING, B.K.; RASKIN, L. Quantification of syntrophic fatty acid-b-oxidizing bacteria in a mesophilic biogas reactor by oligonucleotide probe hybridization. **Applied and Environmental Microbiology**, v.65, p.4767–4774. 1999.

HINDRICHSEN, I.K.; WETTSTEIN, H.R.; MACHMULLER, A.; JÖRG, B.; KREUZER, M. Effect of the carbohydrate composition of feed concentrates on methane emission from dairy cows and their slurry. **Environmental Monitoring and Assessment**, v.107, p.329–350, 2005.

HORN, H.H.V.; WILKIE, A.C.; POWERS, W.J.; NORDSTEDT, R.A. Components of dairy manure management systems. **Journal Dairy Science**, Champaign, v.77,n.7, p.2.008-2.030, 1994.

HUNTER LONG, J.; TAREK, N.; FRANCIS, L.; DE LOS REYES III; JOEL, J. Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. **Process Safety and Environmental Protection**. v.90, p.231–245. 2012.

IEA. International energy agency. Biogas production and utilisation. **IEA Bio energy**. 2005. Available online at: <http://www.iea-biogas.net/Dokumente/Brochure%20final.pdf>

IPCC - Intergovernmental Panel on Climate Change. 2006 **IPCC Guidelines for Greenhouse Gas Inventories**. Japan: Guidelines, 2006.

ITODO, I.N.; AWULU, J.O. Effects of total solids concentrations of poultry, cattle and piggery waste slurries on biogas yield. **Transactions of the ASAE**, St. Joseph, v.42,n.6, p.1.853-1.855, 1999.

KAMIŃSKI, M.; KARTANOWICZ, R.; JASTRZĘBSKI, D.; KAMIŃSKI, M.M. Determination of carbon monoxide, methane and carbon dioxide in refinery hydrogen gases and air by gas chromatography. **Journal of Chromatography A**, v.989, n.2, p.277–283, 2003.

KRAMER, J.K.G.; FELLNER, V.; DUGAN, M.E.R. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. **Lipids**, Heidelberg, v.32, p.1219-1228, 1997.

KÜLLING, D.R.; MENZI, H.; SUTTER, F.; LISCHER, P.; KREUZER, M. Ammonia, nitrous oxide and methane emissions from differently stored dairy manure derived from grass- and hay-based rations, **Nutr. Cycl. Agroecosyst.** (in press), 2002.

KUNZ, A.; BERNDT, A.; FARIAS, A.R.; SCHRAMMEL, B.; ALVES, B.J.R.; GALHARTE, C.A.; AIRA C.; RECZKO, C.A. Gestão ambiental na agropecuária / Julio Cesar Pascale Palhares, Luciano Gebler, editores técnicos. - Brasília, DF: **Embrapa**, 2014. v. 2, p.490. ISBN 978-85-7035-302-3.

LAVREŇČIČ, A.; STEFANON, B.; SUSMEL, P. An evaluation of the Gompertz model in degradability studies of forage chemical components. **Anim Sci**, v.64, p.423-31, 1997.

LUCAS JUNIOR, J, **Algumas Considerações sobre o uso do estrume de suínos como substrato para três sistemas de biodigestores anaeróbios**, 1994. 137f, Tese (Livro Docência Construções Rurais) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, 1994.

MATA-ALVAREZ, J. et al. A critical review on anaerobic co-digestion achievements between 2010 and 2013. **Renewable and Sustainable Energy Reviews**, v.36, p.412-27, 2014.

MOLLER, H.B.; SOMMER, S.G.; AHRING, B.K. Methane productivity of manure, straw and solid fractions of manure. **Biomass Bioenergy**, Oxford, v.26, n.3, p.485-495, 2004.

MURRAY, R.M.; BRYANT, A.M.; LENG, R.A. Rates of production of methane in the rumen and large intestine of sheep. **British Journal of Nutrition**, v.36, p.1-14, 1976.

NEVES, L.; PEREIRA, M.A.; MOTA, M.; ALVES, M.M. Detection and quantification of long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids. **Bioresour. Technol**, v.100, p.91-96, 2009.

NRC, NATIONAL RESEARCH COUNCIL. 2000. **Nutrient Requirements of Beef Cattle**: Seventh Revised Edition: Update 2000. Washington, DC: The National Academies Press. 248 p.

OGEDA, T.L.; PETRI, D.F.S. Biomass Enzymatic Hydrolysis. **Química. Nova**, v.33, p.1549-1558, 2010.

ORRICO, A.C.A.; LOPES, W.R.T.; MANARELLI, D.M.; ORRICO JUNIOR, M.A.P.; SUNADA, N.daS. Co-digestão anaeróbia dos dejetos de bovinos leiteiros e níveis de inclusão de óleo de descarte. **Revista Engenharia Agrícola**, v.36, n.3, p.537-545, 2016.

ORRICO Jr., M.; ORRICO, A. Quantification, characterization, and anaerobic digestion of sheep manure: the influence of diet and addition of crude glycerin. **Environmental Progress & Sustainable Energy**, v.34, p.1038-1043, 2015.

ORRICO JÚNIOR, M.A.P.; ORRICO, A.A., LUCAS JUNIOR, J. Biodigestão anaeróbia dos resíduos da produção avícola: cama de frangos e carcaças. **Revista Engenharia Agrícola**, v.30, n.3, p.546-554, 2010.

ORRICO JÚNIOR, M.A.P.; ORRICO, A.C.A.; LUCAS JÚNIOR, J.; SAMPAIO, A.A.M.; FERNANDES, A.R.M.; OLIVEIRA, E.A.de. Biodigestão anaeróbia dos dejetos da bovinocultura de corte: influência do período, do genótipo e da dieta. **Revista Brasileira de Zootecnia**, v.41,n.6, p.1533-1538, 2012.

OSORIO J.A.S.; VELÁSQUEZ, H.J.C.; GONZÁLEZ, H.S. Evaluación de un sistema de biodigestión en serie para clima frío. **Revista Facultad Nacional de Agronomía Medellín**, v.60, n.2, p.4145-4162. 2007.

PEREIRA, M.A., PIRES, O.C., MOTA, M., ALVES, M.M., Anaerobic biodegradation of oleic and palmitic acids: evidence of mass transfer limitations caused by the accumulation of LCFA onto the anaerobic sludge. **Biotechnology and Bioengineering** 92, 15–23. 2005.

PEREIRA, M.A.; SOUSA, D.Z.; MOTA, M.; ALVES, M.M. Mineralization of LCFA associated to anaerobic sludge: kinetics, transport limitations, enhancement of methanogenic activity and effect of VFA. **Biotechnology and Bioengineering**, v.88, n.4, p.502–510. 2004.

QUEIROZ, S.C. **Função Gompertz aplicada à modelagem de produção do biogás em biodigestores tipo batelada**. 2001. 61p. Dissertação de Mestrado em Agronomia – Faculdade de Ciências Agrônomicas, Universidade Estadual Paulista, Botucatu – SP, 2001

RODRIGUES, J.P.; ORRICO, A.C.A.; ORRICO Jr M.A.P. et al., Adding oil and lipase on the anaerobic digestion of pig manure. **Ciência Rural**, Santa Maria, v.44, n.3, p.544-547, mar, 2014.

SAS. **SAS/STAT-User's guide**, 9.2. Cary, NC, USA: SAS Inst, Inc, 2010.

SOUSSANA, J.F.; TALLEC, T.; BLANFORT, V. Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. **Animal**, v.4, n.3, p.334-530.

SUNADA, N.da; ORRICO, A.C.A., ORRICO Jr, M.A.P.et al. Anaerobic co-digestion of animal manure at different waste cooking oil concentrations. **Ciência Rural**, v.48, n.7, p.e20170517, 2018.

USEPA. (2007). **US Environmental protection agency**. Climate change and greenhouse gas emission. Available on line at: <http://epa.gov/climatechange/emissions/index.html>

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, n.10, p.3583–3597, 1991.

VEDRENNE, F.; BÉLINE, F.; DABERT, P.; BERNET, N. The effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes. **Bioresource Technology, Essex**, v.99, n.1, p.146-155, 2008.

VEYSSET, P.; LHERM, M.; BÉBIN, D. Energy consumption, greenhouse gas emissions and economic performance assessments in French Charolais suckler cattle farms: Model-based analysis and forecasts. **Agricultural Systems**, v.103, p.41-50, 2010.

WENG, C.N.; JERIS, J.S. Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. **Water Res**, v.10, p.9–18, 1976.

WILDENAUER F. X., BLOTEVOGEL, J; WINTER, J. Effect of monensin and 2-bromoethanesulfonic acid on fatty acid metabolism and methane production from cattle manure. **Appl Microbiol Biotechnol.**, v.19, p.125-130, 1984.

YAMULKI, S. Effect of straw addition on nitrous oxide and methane emissions from stored farmyard manures. **Agriculture, ecosystems and environment**, v.112, p.140–145, 2005.