UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

ENERGY SOURCES IN THE SUPPLEMENTATION OF BEEF CATTLE GRAZING *BRACHIARIA BRIZANTHA CV*. XARAÉS

Antônio José Neto

Zootecnista

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Orientadora: Profa. Dra. Telma Teresinha Berchielli

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias - UNESP, Câmpus de Jaboticabal, como parte das exigências para a obtenção do Título de Doutor em Zootecnia

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Câmpus de Jaboticabal

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TÍTULO DA TESE: ENERGY SOURCES IN THE SUPPLEMENTATION OF BEEF CATTLE GRAZING *BRACHIARIA BRIZANTHA CV.* XARAÉS.

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ORIENTADORA: TELMA TERESINHA BERCHIELLI

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Jaboticabal, 18 de fevereiro de 2016

DADOS CURRICULARES DO AUTOR

ANTONIO JOSE NETO – nascido em 10 de março de 1985, na cidade de Campos Belos, Goiás (GO), filho de Almir Eustáquio de Queiroz e Maria das Graças Cardoso Queiroz, ingressou no curso de Zootecnia em Lavras (MG), na Universidade Federal de Lavras (UFLA) em agosto de 2005, graduando-se em fevereiro de 2010. De janeiro a março de 2009 realizou estágio supervisionado no Departamento de Ciência Animal da Escola Superior Agrária do Instituto Politécnico de Bragança - Bragança, Portugal, onde desenvolveu actividades no Laboratório de Qualidade da Carne e da Carcaça, com foco na utilização da ultrassonografia para estimar a composição corporal dos animais, sob orientação do Prof. Dr. Alfredo Jorge Costa Teixeira. Em março de 2010, ingressou no Curso de Pós-Graduação em Ciência Animal, em nível de Mestrado, na Universidade Federal de Mato Grosso (UFMT), campus de Cuiabá (MT), sob orientação do Prof. Dr. Joanis Tilemahos Zervoudakis, obtendo o título de mestre em fevereiro de 2012. Em março de 2012, ingressou no Curso de Pós-Graduação em Zootecnia, em nível de Doutorado, na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho", campus de Jaboticabal (SP), sob orientação da Profa. Dra. Telma Teresinha Berchielli. De setembro de 2014 a julho de 2015 realizou Doutorado - Sanduíche no Agricultural Research and Development Center of Department of Animal Science of University of Nebraska - Lincoln, Nebraska, Estados Unidos, onde acompanhou estudos na área de utilização de subprodutos na dieta de ruminantes sob orientação do Prof. Dr. Galen E. Erickson.

"As conquistas vem quando você cancela as desculpas e transforma as adversidades em determinação."

(autor desconhecido)

"If you have a dream, don't just sit there. Gather courage to believe that you can succeed and leave no stone unturned to make it a reality."

Dr. Roopleen

Dedico

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E t e r n a m e n t e G r a t o!

SUMMARY

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Certificamos que o Protocolo nº 021119/11 do trabalho de pesquisa intitulado **"Balanço de gases de efeito estufa e estratégias de mitigação em pastos de Brachiaria submetidos a diferentes manejos"**, sob a responsabilidade da Profª. Drª. Telma Teresinha Berchielli está de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 07 de Outubro de 2011.

Jaboticabal, 11 de Outubro de 2011.

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ENERGY SOURCES IN THE SUPPLEMENTATION OF BEEF CATTLE GRAZING *BRACHIARIA BRIZANTHA CV.* XARAÉS

ABSTRACT – In the first experiment, one hundred and four (initial BW = 284 ± 38 kg) and sixty (initial BW = 424 ± 34 kg) Nellore bulls were used to evaluate the performance and final carcass characteristics of Nellore bulls during two phases: growing and finishing phase, respectively. The diets used consisted in *Brachiaria brizantha cv.* Xaraés pasture supplemented with two levels of starch, with or without a source of oil. The supplements were corn associated or not with ground soybean (GS) and soybean hulls (SH) associated or not with ground soybean. In relation to animal performance during growing phase, there were no interaction (P = 0.14) between starch level and oil for final BW, ADG, and CrG. However, the addition of oil decreased final BW (P = 0.01), ADG (P < 0.01), total gain (P < 0.01) and CrG (P = 0.01). On the other hand, during finishing phase, there were no interaction (P = 0.11) between starch level and oil for, final BW, ADG, HCW, dressing, CrG, fat depth, and LM area. In contrast, animals supplemented with oil increased final BW (P = 0.01), ADG (P = 0.02), total gain (P = 0.01), HCW (P < 0.01), CrG (P = 0.01), and fat depth (P = 0.04). Furthermore, there was effect of time during growing and finishing phase on values of ADG (P < 0.01) of Nellore bulls. Soybean hulls have a similar energy value to corn when used in supplements to beef cattle on tropical pasture during growing and finish phase. The use of oil supplementation may be effective to reduce enteric CH_4 emissions of Nellore bulls fed *Brachiaria brizantha cv*. Xaraés during the growing and finishing phase, and in addition, may improve performance and final carcass traits of Nellore bulls, only during the finish phase. In another experiment, aimed to evaluate the combined effects of high- or low-starch supplements and oil on intake, digestibility, performance, and methane (CH_4) emissions of growing (n = 44, initial BW = 250.69 ± 27 kg) and finishing (n = 44, initial BW = 414 ± 12 kg) Nellore bulls fed tropical pasture of *Brachiaria brizantha cv.* Xaraés during the rainy and dry season, respectively. Eight animals were slaughtered at a commercial beef plant and served as the reference group at the beginning of each experiment. The other thirty-six animals were distributed in a completely randomized design (three animals per paddock and three paddocks per treatment). The experimental period lasted 133 d, divided into an adaptation period of 21 d and four periods of 28 d each. The supplements were: corn combined with GS; corn without GS; SH combined with GS; and SH without GS. Crude glycerin was used in all supplements to replace (28% of DM) corn or SH. In relation to growing phase, there were no interactions between starch level and oil supplementation on intake of DM (P = 0.67), NDF (P = 0.50), and EE (P = 0.47); on digestibility of DM (P = 0.18) and NDF (P = 0.42); on final BW (P = 0.94), ADG (P = 0.40), FE (P = 0.37); and on CH_4 emissions when expressed in g/d (P = 0.77), kg/yr (P = 0.77), g/kg DMI (P = 0.53). However, independently of starch level utilized, the animals supplemented with oil increased intake of EE (P < 0.01); decreased the digestibility of OM (P = 0.04) and NDF (P = 0.03); and decreased enteric CH_4 emission when corrected for intake of GE (P = 0.04) and EE (P < 0.01). In finishing phase, there were no interactions between starch level and oil supplementation on intake of DM (P = 0.90), NDF (P = 0.65), and EE (P = 0.56); on digestibility of DM (P = 0.12) and

NDF (*P* = 0.12); on final BW (*P* = 0.37), ADG (*P* = 0.41), FE (*P* = 0.47), HCW (*P* = 0.83), dressing (*P* = 0.41), carcass gain (*P* = 0.98), fat depth (*P* = 0.36) and LM area (*P* = 0.91); and on CH_4 emissions when expressed in g/d (*P* = 0.78), kg/yr (*P* = 0.78), g/kg DMI (*P* = 0.81), g/kg ADG (*P* = 0.48), and g/kg of carcass gain (*P* = 0.85). However, independently of starch level utilized, the animals supplemented with oil decreased the digestibility of NDF (*P* = 0.03) and increased EE digestibility (*P* < 0.01); increased the fat depth (*P* = 0.01); and decreased enteric CH_4 emission expressed in g/d (*P* = 0.04) and kg/yr (*P* = 0.04), and when was corrected to GE (*P* = 0.02) and EE (*P* < 0.01) intake, and ADG (*P* = 0.02) for animals fed high- or low-starch supplement. In growing or finishing phase, soybean hulls have the similar estimated feeding value to that of corn, and the use of oil supplementation may be effective to reduce enteric methane emission of Nellore bulls grazing tropical pasture. Regarding the study of cannulated animals, the objective was evaluate the effect of oil supplementation combined with high- or low-starch on intake, nutrient digestibility, rumen fermentation parameters, and rumen microbial profile of young Nellore steers grazing *Brachiaria brizantha cv.* Xaraés during two phases: growing and finishing. In the growing phase, eight ruminal cannulated Nellore steers (424.8 kg ± 35.5) at 20 mo of age were used in a replicate 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments (high or low starch, with or without a source of oil) and an experimental period of 21 d. The supplements were corn combined with GS; corn without GS; SH combined with GS; and SH without GS. Animals were supplemented at the rate of 500 g/100kg BW. There were no interactions between starch level and oil supplementation on DM and nutrients intake (*P* > 0.01). The addition of oil decreased the intake of DM (*P* = 0.01), forage DM (*P* < 0.01), OM (*P* = 0.01), CP (*P* = 0.02), NDF (*P* < 0.01), and GE (*P* = 0.01), independently of starch level used. Animals fed with low-starch and without oil had greater digestibility of DM (*P* < 0.01), OM (*P* < 0.01), CP (*P* < 0.01), NDF (*P* = 0.01), and GE (*P* = 0.01) than animals fed with other supplements. The addition of oil in the supplements decreased the pH (*P* = 0.02) and NH_3-N (*P* = 0.02); and decreased the numbers of Dasytricha (*P* < 0.01), Isotricha (*P* < 0.01), and total protozoa (*P* < 0.01). The percentage of *Ruminococcus albus* (*P* = 0.0003), *Ruminococcus flavefaciens* (*P* = 0.0002), and *Archeas* (*P* < 0.0004) were higher for low-starch without oil diets than for other diets. Additionally, animals supplemented with oil decreased the number of *Fibrobacter succinogenes* (*P* = 0.0003), independently of starch level used. Oil supplementation reduce intake, protozoa population, and fibrolytic rumen bacteria. The use of low-starch supplementation without oil may be effective to increase digestibility of DM and nutrient, and *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Archeas* population in the rumen of growing Nellore steers grazing tropical pasture. In the finishing phase, eight ruminal cannulated Nellore steers (514.5 kg ± 30.1) at 24 mo of age were used in a replicate 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments (high or low starch, with or without a source of oil) and an experimental period of 21 d. The supplements were corn combined with GS; corn without GS; SH combined with GS; and SH without GS. Animals were supplemented at the rate of 1000 g/100 kg BW. There were no interactions between starch-based supplementation level and oil with regard to DM and nutrients intake (*P* > 0.05). Animals supplemented with low starch and no oil showed greater (10.77%) digestibility of CP (*P* = 0.01) than those supplemented with high-starch and no oil. Total apparent digestibility of DM (P < 0.01), OM (*P* < 0.01), NDF (*P* = 0.03), and GE

(*P* = 0.02) decreased with oil supplementation. There were no interactions between starch × oil for pH, NH_3-N, and total VFA (*P* > 0.05). Animals supplemented with oil showed lower acetate production (*P* < 0.01) than those supplemented without oil, independent of starch level. The addition of oil in the supplements decreased the population of Dasytricha (*P* < 0.01), Polyplastron (*P* < 0.21), and Diploplastron (*P* = 0.04). Supplementing the animals with low-starch without oil increased the numbers of *Ruminococcus albus* compared with the other supplements (*P* = 0.0120). There was also interaction between starch × oil for *Selenomonas ruminantium* (*P* = 0.0003), once low-starch supplement, with or without oil, decreased the number of *Selenomonas ruminantium* of Nellore steers. The addition of oil in the supplements decreased the number of *Fibrobacter succinogenes* (*P* < 0.0001), *Ruminococcus flavefasciens* (*P* < 0.0001), and *Archeas* (*P* < 0.0001), but increased of *Anaerovibrio lipolytica* (*P* < 0.0001), independently of starch level used. Oil supplementation decreases the intake, digestibility, acetate production, protozoa population, and fibrolytic rumen bacteria. The use of soybean hulls without oil supplementation may be effective to increase digestibility of CP, and *Ruminococcus albus* of finishing Nellore steers grazing *Brachiaria brizantha cv.* Xaraés during the dry season. The last study evaluated the fatty acid intake, fatty acid composition, and meat quality traits of 60 young Nellore bulls fed diets with two levels of starch-based supplement with or without a source of oil (ground soybean; GS). The supplements were corn without GS, corn associated with GS, soybean hulls (SH) without GS, and SH associated with GS. There were interaction between starch-based supplementation level and oil to intake of vaccenic (*P* < 0.01), linoleic (*P* < 0.01), Total PUFA (*P* = 0.01). Meat from animals supplemented with-high starch and without oil increased the percentage of vaccenic acid (*P* = 0.01). The use of low-starch supplements with oil increases intake of linoleic and total PUFA. Starch-based or oil supplementation not affect the myristic or palmitic acid content in the *longissimus dorsi* muscle. Oil supplementation increases the level of stearic acid and the n-6/n-3 ratio, but decreases the percentage of linolenic acid in muscle of Nellore bulls grazing *Brachiaria brizantha* cv. Xaraés during finishing phase.

Keywords: fatty acid, glycerol, methane, Nellore, oil, performance

FONTES DE ENERGIA NA SUPLEMENTAÇÃO DE BOVINOS DE CORTE EM PASTAGENS DE *BRACHIARIA BRIZANTHA* CV. XARAÉS

RESUMO – No primeiro experimento, foram utilizados cento e quatro (PC inicial = 284 ± 38 kg) e sessenta (PC inicial = 424 ± 34 kg) tourinhos da raça Nelore com o objetivo de avaliar os efeitos de suplementos com alto ou baixo amido, associados ou não com uma fonte de óleo, sobre o desempenho e características finais da carcaça durante duas fases: recria e terminação, respectivamente. Os suplementos utilizados foram milho sem soja grão moída (SG), milho associado com SG, casca de soja (CS) sem SG, e CS associada com SG. The experimental design was completely randomized in a 2 × 2 factorial arrangement (high or low starch, with or without a source of oil). Each paddock was considered the individual experimental unit. Em relação ao desempenho dos animais durante a fase de recria, não houve interação (P = 0,14) entre o nível de amido e óleo para o PC final, GMD e ganho de carcaça. No entanto, a adição de óleo diminuiu o PC final (P = 0,01), GMD (P < 0,01), o ganho total (P < 0,01) e ganho de carcaça (P = 0,01). Por outro lado, durante a fase de terminação, não houve interação (P = 0,11) entre o nível de amido e óleo para PC final, GMD, PCQ, rendimento de carcaça, ganho de carcaça, espessura de gordura subcutânea e AOL. No entanto, os animais suplementados com óleo aumentaram o PC final (P = 0,01), GMD (P = 0,02), ganho total (P = 0,01), PCQ (P < 0,01), o ganho de carcaça (P = 0,01) e a espessura de gordura (P = 0,04). Além disso, houve efeito do tempo durante a fase de recria e terminação para os valores de GMD (P < 0,01) dos tourinhos da raça Nelore. A casca de soja tem um valor energético semelhante ao milho quando usado em suplementos para bovinos de corte em pastagem tropical durante a fase de recria e terminação. O uso de suplementação com óleo pode ser eficaz para melhorar o desempenho e as características finais da carcaça de tourinhos da raça Nelore em pastagens de *Brachiaria brizantha cv.* Xaraés, somente durante a fase de terminação. No outro experimento, objetivou-se avaliar os efeitos de suplementos com alto ou baixo amido, associados ou não com óleo, sobre o consumo, a digestibilidade, desempenho e emissões de metano (CH_4) da fase de recria (n = 44, PC inicial = 250,69 ± 27 kg) e terminação (n = 44, PC inicial = 414 ± 12 kg) de tourinhos da raça Nelore alimentados com pasto tropical de *Brachiaria brizantha cv.* Xaraés durante a estação das águas e da seca, respectivamente. Oito animais foram abatidos em uma planta de frigorífico comercial de bovinos e serviu como grupo de referência no início de cada experimento. Os outros trinta e seis animais foram distribuídos em um delineamento experimental inteiramente casualizado (três animais por piquete e três piquetes por tratamento). O período experimental foi de 133 dias, divididos em um período de adaptação de 21 dias e quatro períodos de 28 dias cada. Os suplementos foram milho sem soja grão moída (SG), milho associado com SG, casca de soja (CS) sem SG, e CS associada com SG. Glicerina bruta foi utilizada em todos os suplementos para substituir (28% da MS) do milho ou da CS. Em relação à fase de recria, não houve interações entre nível de amido e suplementação com óleo sobre o consumo de MS (P = 0,67), FDN (P = 0,50) e EE (P = 0,47); sobre a digestibilidade da MS (P = 0,18) e do FDN (P = 0,42); sobre o PC final (P = 0,94), GMD (P = 0,40), e na EA (P = 0,37); e sobre as emissões de CH_4 quando expressa

em g/dia (*P* = 0,77), kg/ano (*P* = 0,77), g/kg CMS (*P* = 0,53). No entanto, independentemente do nível de amido utilizado, os animais suplementados com óleo aumentaram o consumo de EE (*P* < 0,01); diminuíram a digestibilidade MO (*P* = 0,04) e FDN (*P* = 0,03); e também, reduziram a emissão CH_4 entérica quando corrigido para consumo de EB (*P* = 0,04) e EE (*P* < 0,01). Na fase de terminação, não houve interações entre nível de amido e suplementação com óleo sobre o consumo de MS (*P* = 0,90), FDN (*P* = 0,65) e EE (*P* = 0,56); sobre a digestibilidade da MS (*P* = 0,12) e FDN (*P* = 0,12); no PC final (*P* = 0,37), GMD (*P* = 0,41), EA (*P* = 0,47), PCQ (*P* = 0,83), rendimento de carcaça (*P* = 0,41), ganho de carcaça (*P* = 0,98), espessura de gordura (*P* = 0,36) e na AOL (*P* = 0,91); e sobre a emissão de CH_4 quando expressa em g/dia (*P* = 0,78), kg/ano (*P* = 0,78), g/kg de CMS (*P* = 0,81), g/kg GMD (*P* = 0,48), e em g/kg de ganho de carcaça (*P* = 0,85). No entanto, independentemente do nível de amido utilizado, os animais suplementados com óleo diminuíram a digestibilidade da FDN (*P* = 0,03) e aumentaram a digestibilidade do EE (*P* < 0,01); aumentaram a espessura de gordura subcutânea (*P* = 0,01); e reduziram a emissão de CH_4 entérico, expresso em g/dia (*P* = 0,04) e kg/ano (*P* = 0,04), e quando foi corrigido para consumo de EB (*P* = 0,02) e de EE (*P* < 0,01), e GMD (*P* = 0,02) para os animais suplementados com alto ou baixo amido. Em relação aos estudos de parâmetros ruminais, o objetivo foi avaliar o efeito da suplementação com óleo combinado com alto ou baixo amido sobre o consumo, a digestibilidade dos nutrientes, parâmetros de fermentação ruminal e o perfil dos microrganismos no rúmen, de novilhos da raça Nelore em pastagem de *Brachiaria brizantha* cv. Xaraés durante duas fases: recria e terminação. Na fase de recria, oito animais da raça Nelore, canulados no rúmen (424,8 kg ± 35,5) e com 20 meses de idade foram usados em um quadrado latino duplo 4 × 4, com um arranjo fatorial 2 × 2 dos tratamentos (alto ou baixo amido, com ou sem fonte de óleo) e um período experimental de 21 dias. Os suplementos foram milho sem soja grão moída (SG), milho associado com SG, casca de soja (CS) sem SG, e CS associada com SG. Os animais foram suplementados à taxa de 500 g/100 kg do peso corporal. Não houve interações entre nível de amido e suplementação com óleo sobre o consumo de MS e nutrientes (*P* > 0,01). A adição de óleo diminuiu o consumo de MS (*P* = 0,01), forragem (*P* < 0,01), MO (*P* = 0,01), PB (*P* = 0,02), FDN (*P* < 0,01) e EB (*P* = 0,01), independentemente do nível de amido utilizado. Animais alimentados com baixo amido e sem óleo apresentaram maior digestibilidade da MS (*P* < 0,01), MO (*P* < 0,01), PB (*P* < 0,01), FDN (*P* = 0,01) e EB (*P* = 0,01) do que os animais alimentados com outros suplementos. A adição de óleo nos suplementos diminuiu o pH (*P* = 0,02), NH_3-N (*P* = 0,02), número de Dasytricha (*P* < 0,01), Isotricha (*P* < 0,01) e total de protozoários (*P* < 0,01). A percentagem de *Ruminococcus albus* (*P* = 0,0003), *flavefaciens Ruminococcus* (*P* = 0,0002), e *Archeas* (*P* < 0,0004) foram maiores para as dietas com baixo amido e sem óleo, do que para outras dietas. Além disso, os animais suplementados com óleo diminuiram o número de *Fibrobacter succinogenes* (*P* = 0,0003). Suplementação com óleo reduz o consumo, a população de protozoários e as bactérias fibrolíticas no rúmen. O uso de suplementos com baixo amido e sem óleo pode ser eficaz para aumentar a digestibilidade da MS e dos nutrientes, e também aumentar a população de *Ruminococcus albus*, *Ruminococcus flavefaciens*, e *Archeas* no rúmen de novilhos Nelore em pastejo, durante a fase de recria. Na fase de terminação, oito animais da raça Nelore, canulados no rúmen (514,5 kg ± 30,1) e com 24 meses de idade foram

usados em um quadrado latino duplo 4 × 4, com um arranjo fatorial 2 × 2 dos tratamentos (alto ou baixo amido, com ou sem fonte de óleo) e um período experimental de 21 dias. Os suplementos foram milho sem soja grão moída (SG), milho associado com SG, casca de soja (CS) sem SG, e CS associada com SG. Os animais foram suplementados à taxa de 1000 g/100 kg de peso corporal. Não houve interações entre o nível de amido e óleo na suplementação em relação ao consumo de MS e nutrientes ($P > 0,05$). Animais suplementados com baixo amido e sem óleo apresentaram maior (10,77%) digestibilidade da PB ($P = 0,01$) do que aqueles suplementados com alto amido e sem óleo. A digestibilidade aparente total da MS ($P < 0,01$), MO ($P < 0,01$), FDN ($P = 0,03$) e EB ($P = 0,02$) diminuiu com uso do óelo na suplementação. Não houve interações entre amido × óleo para pH, N-NH_3 e total de AGV ($P > 0,05$). Animais suplementados com óleo apresentaram uma menor produção de acetato ($P < 0,01$) do que aqueles suplementados sem óleo, independente do nível de amido. A adição de óleo nos suplementos diminuiu a população de Dasytricha ($P < 0,01$), Polyplastron ($P < 0,21$), e de Diploplastron ($P = 0,04$). Suplementando os animais com baixo amido e sem óleo, aumentou o número de *Ruminococcus albus* em comparação com os outros suplementos ($P = 0,0120$). Houve também uma interação entre amido × óleo para *Selenomonas ruminantium* ($P = 0,0003$), uma vez que, os suplementos com baixo amido, com ou sem óleo, diminuiram o número de *Selenomonas ruminantium* dos novilhos Nelore. A adição de óleo nos suplementos diminuiu o número de *Fibrobacter succinogenes* ($P < 0,0001$), *Ruminococcus flavefasciens* ($P < 0.0001$), e de *Archeas* ($P < 0.0001$), mas aumentou o de *Anaerovibrio lipolytica* ($P < 0.0001$). Suplementação com óleo reduziu o consumo, a digestibilidade, a produção de acetato, a população de protozoários e bactérias ruminais fibrolíticas. O uso da suplementação com casca de soja e sem óleo pode ser eficaz para aumentar a digestibilidade da PB, e a população de *Ruminococcus albus* de novilhos Nelore em pastagens de *Brachiaria brizantha* cv. Xaraés durante a fase de terminação. O último estudo avaliou a ingestão de ácidos graxos, composição de ácidos graxos e características de qualidade da carne de 60 tourinhos da raça Nelore alimentados com dietas com dois níveis de amido, com ou sem uma fonte de óleo (grão de soja moída; SG). Os suplementos foram milho sem soja grão (SG), milho associado com SG, casca de soja (CS) sem SG, e CS associada com SG. Houve interação entre o nível de amido e óleo sobre consumo de ácido vacénico ($P < 0,01$), linoleico ($P < 0,01$) e total PUFA ($P = 0,01$). A carne dos animais suplementados com alto amido e sem óleo aumentou a porcentagem de ácido vacênico ($P = 0,01$). O uso de suplementos com baixo amido e com óleo aumenta a ingestão de ácido linoléico e PUFA total. Suplementação à base de amido ou óleo não afeta o teor de ácido mirístico ou palmítico no músculo *Longissimus dorsi*. Suplementação com óleo aumenta o nível de ácido esteárico e a relação n-6/n-3, mas diminui a percentagem de ácido linolênico no músculo de tourinhos Nelore em pastagem de *Brachiaria brizantha* cv. Xaraés durante a fase de terminação.

Palavras-chave: ácidos graxos, desempenho, glicerol, metano, Nelore, óleo

CHAPTER 1 - GENERAL CONSIDERATIONS

Projections indicate that the world population may increase by 1 billion over the next 12 years and reach 9.6 billion by 2050 (UN, 2013). Population growth and also the change seen in diet composition related to increased welfare levels (e.g. ALEXANDRATOS et al., 2006; VINNARI and TAPIO, 2009), with increased demand for animal products in developing countries, will increase future demand and natural resources utilization has been of critical interest to researchers, especially about the use of water, land and fertilizers (VAN HAM et al., 2013; LEACH et al., 2012).

In 2005, agriculture occupied about 38% of the global land area yielding an average agricultural land endowment of 0.76 ha per capita. Without technical progress and agricultural intensification and with current rates of population growth, agriculture would need an area equivalent to one half and two-third of the current terrestrial land area by 2030 and 2070, respectively, in order to maintain current food consumption levels per capita (FAO, 2011).

The global food system is experiencing profound changes as a result of anthropogenic pressures, including demographic and dietary changes, climate change, bioenergy development and natural resource constraints. These and related forces are also driving structural changes in the livestock sector, which has developed as one of the most dynamic parts of the agricultural economy (FAO, 2009).

Global consumption of dairy products and beef is projected to rise by well over 50% by 2050 (FAO, 2011). World meat consumption increased from 47 million tons in 1950 to 260 million tons in 2005, more than doubling the consumption per person from 17 to 40 kg/year (BROWN, 2006). In the face of competing demands for resources, ruminants play a key role in human food production in converting plant resources that humans cannot consume into high-quality food that humans can eat. The ability of ruminants to turn fibrous feed resources into edible animal food of high biological value is likely to become of greater significance in terms of global human food production as the population of the planet and demand for human-edible plant resources increases rapidly (DIJKSTRA et al., 2013).

Increased global demand for beef is being met from two main sources: rapidly expanding feedlot production; and intensification and spatial expansion of managed grazing systems. Both have significant regional and global environmental impacts (McALPINE et al., 2009). In Brazil, the majority of agricultural land in use is covered with pastures, almost 159 million ha, however most of it with low yields (IBGE, 2011). By allowing a fraction of current pasture area to accommodate the expansion of food and biofuel crops, intensification of existing pastoral systems is a strategy to avoid further loss of native vegetation. Thus increased animal production efficiency makes this a real possibility.

The area of pastureland dedicated to beef production in Brazil decreased from 174.5 Mha in 1980 to 159.8 Mha in 2006 (IBGE, 2011). In this same period meat production increased from 2.09 Mt to 6.89 Mt, following the increase in productivity from 11.9 to 43.4 kg/ha of carcass equivalent (MARTHA et al., 2012). The observed increase in animal performance further contributed to lower methane emission intensity (i.e. methane emissions per product unit). The land-saving effect due to productive gains in Brazilian beef sector was of 525 million hectares in the 1950 - 2006 period. But there is much room for improvement because the average Brazilian stocking rate was just 1.08 head/ha (MARTHA et al., 2012).

Increasing the efficiency of ruminant production is vital for food production to meet increasing demands. As livestock production grows and intensifies, it depends less and less on locally available feed and increasingly on feed concentrates. There is a shift from the use of low-quality roughages (crop residues and natural pasture) towards high-quality agro-industrial byproducts and concentrates (FAO, 2009).

In this sense, there is a new competition pattern, characterized by better adaptation of livestock due to strong demand, replacing the widespread availability of conventional products. A consequence of this new pattern of competition in the beef cattle industry is the use of agro-industrial byproducts in the diet of these animals, with the main objective to reduce production costs and, in addition, increase productivity, restore degraded areas, add income to the bio-energy production chain and reduce their environmental liabilities, providing appropriate conditions for future generations (MITLOEHNER, 2014).

This means that cereals can be largely replaced by these byproducts (MIRZAEI-AGHSAGHALI et al., 2011) and competition between human and animal nutrition can be decreased. Consequently, byproducts are becoming increasingly important in the food and fiber chain, because they are available for use as cattle feed at competitive prices compared to other commodities.

Crude glycerin is a byproduct of biodiesel agroindustry resulting from the formation of methyl esters of fatty acids from triglycerides. This byproduct has been reported as a potential energy source to partially replace starch-based ingredients in the diet, such as corn, because glycerol (an 85% constituent of crude glycerin) is converted to propionate in the rumen, decreasing the acetate:propionate ratio, and acts as a precursor for hepatic glucose synthesis (ABO-EL NOR et al., 2010; ABUGHAZALEH et al., 2011; AVILA et al., 2011; RAMOS and KERLEY, 2012).

Most research evaluating crude glycerin in ruminants has been based on performance measurements such as DMI, ADG, and G:F. The results show that glycerin can be used in ruminant diets up to 10% of diet DM without compromise intake and performance (DROUILLARD, 2012; PARSONS et al., 2009; SCHRODER and SUDEKUM, 2009). Hales et al. (2013) reported that propionate concentration was greatest in the diet with 10% glycerin at multiple time points postfeeding. Furthermore, earlier research has demonstrated that ruminal propionate concentrations were greater when a 45 *vs.* 0, 15, or 30% glycerin diet was added to continuous culture fermenters in place of corn (ABUGHAZALEH et al., 2011). If glycerin is primarily converted to propionate in the rumen, it is thought that glycerin may potentially decrease enteric methane emission and increase retained energy in the animal.

Globally, methane emissions account for 40% to 45% of greenhouse gas emissions from ruminant livestock, with over 90% of these emissions arising from enteric fermentation (FAO, 2006). Cattle are an important source of methane in many countries (e.g., Brazil) because of their large population and high CH_4 emission rate due predominantly of enteric fermentation and to a lesser extent, from manure storage. Although enteric fermentation is essential for the effective degradation of organic matter in the rumen (McALLISTER et al., 1996; BEAUCHEMIN et al., 2009), CH_4 has no nutritional value to the host and therefore represents a loss of up to 12%

of gross energy intake (JOHNSON and JOHNSON, 1995; BEAUCHEMIN et al., 2008).

Reduction of carbon dioxide to CH_4 is critical for efficient ruminal fermentation because it prevents the accumulation of reducing equivalents in the rumen (McALLISTER et al., 2015). Methane generated in the rumen is formed from hydrogen produced during the fermentation, and is formed by a group of microbes called methanogens, which form a subgroup of the domain Archaea (HUNGATE, 1967). Methane provides an essential means of H_2 removal, as H_2 in the rumen can inhibit hydrogenase activity and limit the oxidation of sugar if alternative means of H_2 disposal are unavailable (McALLISTER and NEWBOLD, 2008). Although CH_4 emission appears essential for efficient ruminal digestion, emissions also present an environmental concern because CH_4 is a potent greenhouse gas with a global warming potential (over 100 yr) 28 times that of CO_2 (IPCC, 2013).

Hydrogen is the most important energy source for methane-producing methanogens in the rumen, although formate (and to a limited extent, methanol) are also produced and used by methanogens. Different methanogen species use H_2 to reduce CO_2 to methane sequentially via a number of very similar pathways containing enzymes and co-factors not found in non-methanogens (THAUER et al., 2008).

The activity of the H_2-consuming methanogens in the rumen reduces the H_2 concentration to low levels, which allows the primary fermentation of the feed to proceed more rapidly. This means that the animal gains more fatty acids within a given time (WOLIN, 1979). High concentrations of H_2 in the rumen are expected to slow the activity of the microbes that ferment the feed, potentially slowing conversion to fatty acids (McALLISTER and NEWBOLD, 2008), and co-cultivation of H_2-producing and H_2-utilising microbes result in a more rapid fermentation (REES et al., 1995; MORVAN et al., 1996).

The primary factors affecting enteric methane emissions are the quantity and quality of the diet. As forage fiber content increases, nutrient digestion and passage rate decrease, increasing the predominate ruminal fermentation pathways and subsequent enteric CH_4 emission (CHIAVEGATO et al., 2015). Grazing management is a combination of several factors, such as stocking rate, density, and rest periods.

These factors define the relationship between herbage supply (ANIMUT et al., 2005) and forage quality, thus influencing herbage utilization efficiency, animal performance, and production per hectare (PINARES-PATIÑO et al., 2007).

According to classic study of Blaxter and Clapperton (1965), increased intake of poor-quality, less-digestible preserved forages have little effect on CH_4 emission when expressed on a DM intake basis. However, for feeds with higher digestibility, increased DM intake depresses the amount of CH_4 produced per unit of feed consumed (HAMMOND et al., 2009, HAMMOND et al., 2013). Cattle fed high- to moderate-quality forages lose 6.5% of GE intake as CH_4, while those fed high-grain diets may only lose 3% of GE intake as CH_4 (IPCC, 2006).

Therefore, grazing management may be used as a potential mitigant through grazing forages at the optimal maturity for increasing forage quality, allowing for adequate pregrazing herbage mass or intensive grazing (BEAUCHEMIN et al., 2008). The impact on CH_4 mitigation, when scaled per unit of animal product, should be typically greater when animals consume higher quality forage. Increasing quality or digestibility of forages will increase production efficiency and this will likely result in decreased CH_4 produced per unit of product (e.g., methane emission per kg of meat).

Thus, strategies that mitigate CH_4 emissions are not only environmentally beneficial, but also result in greater energy-use efficiency of feed by the animal. Bruinenberg et al. (2002) and Nkrumah et al. (2006) reported that a 25% reduction in CH_4 emissions could potentially increase body weight gain of growing cattle by 75 g/d based on the energy balances. Given the increasing demand for high-protein foods, emerging mitigation strategies will likely focus on reducing emissions per unit of animal product and not to reducing overall greenhouse gas emissions.

Consequently, significant effort has been directed toward improving our understanding of ruminal CH_4 formation and the identification of strategies that reduce methanogenesis (HRISTOV et al., 2013; KNAPP et al., 2014). Methane mitigation is effective in one of two ways: either a direct effect on the methanogens or an indirect effect caused by the impact of the strategy on substrate availability for methanogenesis, usually through an effect on the other microbes of the rumen (HOOK et al., 2010).

The components of ruminant diet, especially type of carbohydrate, are important for methane emission as they are able to influence the ruminal pH and subsequently alter the microbiota present (JOHNSON and JOHNSON, 1995). The digestibility of cellulose and hemicellulose are strongly related to methane emission, more so then soluble carbohydrate (HOLTER and YOUNG, 1992).

Carbohydrates are the major source of energy for rumen microorganisms and the single largest component (60-70%) of the ruminant diet. There are two broad classifications of CHO: the fibrous consist of elements found in the plant cell wall) and the nonfibrous (located inside the cells of plants and are usually more digestible than the fibrous CHO). However, even though pectin is a part of the cell wall, it is considered a nonfibrous CHO because compared to hemicellulose, the rumen microorganisms completely and rapidly ferment the pectin (NRC, 2001; ISHLER and VARGA, 2001).

Corn grains are used for food, feed, and the growing bio-fuel industry. This competition for corn has tightened the supply that is available for livestock feed and, as a result, has increased feed costs. The partial replacement of starch with cost-effective, low-starch, nonforage fiber sources represents a potential alternative to help overcome these issues. Previous research conducted on partial substitution of starch with nonforage fiber sources such as soybean hulls has led to the replacement of significant portions of starch from beef cattle diets (IPHARRAGUERRE et al., 2002; AIKMAN et al., 2006; JOSE NETO et al., 2015).

Soybean hulls, ingredient rich in pectin used in the diet of ruminants has a feeding value comparable with corn (HIBBERD et al., 1987; ANDERSON et al., 1988), and in some cases have alleviated adverse impacts on forage utilization often exhibited by cereal grains (KLOPFENSTEIN and OWEN, 1988; MARTIN and HIBBERD, 1990; GALLOWAY et al., 1993). Beneficial effects conferred by supplementation and supplementation type (starch-based vs. fiber based systems) can be altered by the quality of forage in the diet.

On the other hand, the starch component of the diet is also known to promote propionate formation, through a shift to amylolytic bacteria, and a reduction in ruminal pH, leading to a decrease in methanogenesis (VAN KESSEL and RUSSELL, 1996).

Starch is a polymer of glucose linked to another one through the glycosidic bond, which can be cleaved by enzymes. Two types of glucose polymers are present in starch: amylose and amylopectin. Amylose is a linear polymer consisting of up to 6000 glucose units with α-1,4 glycosidic bonds. Amylopectin consists of short α-1,4 linked to linear chains of 10–60 glucose units and α -1,6 linked to side chains with 15-45 glucose units (DEATHERAGE et al., 1955). However, starch is packaged in granules that are embedded in a protein matrix in the seed endosperm, which varies in solubility and resistance to digestion (KOTARSKI et al., 1992).

The extent and location of digestion of dietary carbohydrates affects the contribution to the energy or nutrient supplies to the animal, which alter types and amounts of products made available to the animal. In addition, altering the concentration and ruminal fermentability of starch in rations affects digestibility of starch (NGONYAMO-MAJEE et al., 2008), ruminal pH and fiber digestibility (FIRKINS et al., 2001), and the type, amount, and temporal absorption of fuel (e.g., acetate, propionate, lactate, glucose) available to ruminants (ALLEN, 2000).

The need to improve and optimize the efficiency of starch digestion is an important research focus in animal nutrition in ruminants a large proportion of starch from feed grains is fermented by microorganisms in the rumen, but a substantial amount of starch can be digested in the small intestine or fermented in the large intestine (OFFNER et al., 2003; FOLEY et al., 2006). Carbohydrates digested in the small intestine provide monosaccharides, notably glucose from starch. Ruminally, products of fermentation are much more diverse and come in the forms of gases, organic acids, and microbial mass (HALL and EASTRIDGE, 2014). Thus dietary starch fractions may be termed by site of digestion as ruminal fermentable starch, ruminal bypass starch, undigestible starch, and ruminally resistant starch (DECKARDT et al., 2013).

Research studies have examined ways to modulate the rumen degradability of starch, aiming to improve ruminant feed efficiency by changing the nature and amount of starch available to rumen microbiota, and/or shifting some starch digestion to the small intestine to improve its energetic efficiency, and moreover reduce enteric methane emission (HARMON et al., 2004; HUNTINGTON et al., 2006).

Compared with dietary fiber, starch fermentation in the rumen may result in reduced enteric CH_4 emission because fermentation of starch favors production of propionate (BANNINK et al., 2006), creating an alternative hydrogen sink to methanogenesis. Moreover, unlike fiber and sugar, a substantial fraction of potentially fermentable starch may escape from rumen fermentation to be digested enzymatically in the small intestine, adding to the energy supply of the animal without associated losses of energy with CH_4 emission (DIJKSTRA et al., 2011).

Another nutritional strategy to mitigate methane emissions from ruminants is the use of lipids. Inclusion of lipids in the diet decreases the amount of OM that is fermented in the rumen and therefore, reduces CH_4 emission (MACHMÜLLER et al., 2000; BEAUCHEMIN et al., 2009; MARTIN et al., 2010). Additionally, lipids can exhibit direct inhibitory effects on methanogens and protozoa (MACHMÜLLER et al., 2000; BEAUCHEMIN et al., 2009; MARTIN et al., 2010).

Numerous mitigation strategies have been investigated, each with varying impacts and differing durations of altering rumen microbial populations. Dietary strategies are most commonly employed, of which the most effective approach to inhibiting ruminal methanogenesis is inclusion of dietary medium- and long-chain saturated fatty acids, acting as a hydrogen sink through the hydrogenation of fatty acids (JOHNSON and JOHNSON, 1995; MACHMÜLLER et al., 2003; ZHOU et al., 2013).

Henderson (1973) reported that growth of M. *ruminantium* was inhibited by the addition of unsaturated and saturated medium-chain (C_{12} to C16) fatty acids. Further studies reported lauric acid (C_{12}) decreases cell viability of M. *ruminantium* (ZHOU et al., 2013). Therefore, the addition of fats to ruminant diets has also been recommended, as it similarly increases energy efficiency and hence reduces methanogenesis. However, greater concentrations of fats decrease methane production substantially, they often exert detrimental effects on fiber digestion, and consequently animal performance (PATRA, 2013).

In this sense, limited information is available on combined effects of different carbohydrate forms and oil source on animal performance and enteric CH_4 emissions from beef cattle on tropical pasture. The hypothesis of the present study is that soybean hulls could replace corn as a source of energy, and fat supplementation

could reduce enteric CH_4 emissions without affecting performance of young Nellore bulls.

The objective of this study was to evaluate the combined effects of high- or low-starch supplements and oil on intake, digestibility, performance, carcass characteristics, and methane emissions of young Nellore bulls fed tropical pasture of *Brachiaria brizantha cv*. Xaraés during the growing and finishing phase.

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CHAPTER 2

O artigo a seguir está redigido conforme normas de publicação do *Journal of Animal Science* exceto o posicionamento das tabelas.

PASTURE CHARACTERISTICS AND PERFORMANCE OF NELLORE BULLS FED WITH TWO LEVELS OF STARCH-BASED SUPPLEMENT WITH OR WITHOUT OIL ON GROWING AND FINISHING PHASES

ABSTRACT: The objective of this study was evaluate the effects of two levels of starch-based supplement with or without oil on performance and final carcass characteristics of Nellore bulls during two phases: growing (n = 104, initial BW = 284 ± 38 kg and age of 15 mo) and finish (n = 60, initial BW = 424 ± 34 kg and age of 20 mo), grazing tropical pasture (*Brachiaria brizantha cv.* Xaraés). The experimental design was completely randomized in a 2 × 2 factorial arrangement (high or low starch, with or without a source of oil). In relation to chemical composition of forage, there were no main effects of starch level or oil, or interactions between starch level and oil supplementation with regard to OM, NDF, iNDF, CP, GE, and EE among different paddocks during growing ($P = 0.15$) and finishing phase ($P = 0.07$). However, there was effect of evaluation time ($P < 0.01$) on chemical composition of forage at total experimental period. There were no main effects of starch level or oil, or interaction between starch × oil for total forage mass, dead mass, leaf mass, stem mass, leaf/stem ratio, height, and stocking rate of pasture during growing ($P = 0.24$) and finishing phase ($P = 0.09$). However, there was effect of evaluation time ($P < 0.01$) on values of percentage of forage components, height and stocking rate during experimental period. There were no interactions between starch level and oil supplementation on fractions of carbohydrate A + B_1, B_2 and C, and for fractions of protein A , B_1 + B_2, B_3 and C of *Brachiaria brizantha* cv. Xaraés during growing ($P = 0.37$) and finishing phase ($P = 0.12$). However, there was effect of evaluation time ($P < 0.01$) on values of carbohydrate and protein fractions of forage during different season. In relation to animal performance during growing phase, there were no interaction ($P = 0.14$) between starch level and oil for initial BW, final BW, ADG, total gain, and carcass gain (CrG). However, the addition of oil decreased final BW ($P = 0.01$), ADG ($P < 0.01$), total gain ($P < 0.01$) and CrG ($P = 0.01$). In addition, animals supplemented with high starch decreased CrG ($P < 0.01$). On the other hand, during finishing phase, there were no interaction ($P = 0.11$) between starch level and oil for initial BW, final BW, ADG, total gain, HCW, dressing, CrG, fat depth, and LM area. In contrast, animals supplemented with oil increased final BW ($P = 0.01$), ADG ($P = 0.02$), total gain ($P = 0.01$), HCW ($P < 0.01$), CrG ($P = 0.01$), and fat depth ($P = 0.04$). Furthermore, there was effect of evaluation time during growing and finishing phases on values of ADG ($P < 0.01$) of

Nellore bulls. High or low-starch supplements have a same feeding value to Nellore bulls grazing tropical pasture during growing and finish phase. Animals supplemented with low-starch incresead carcass gain in the growing phase. The use of oil supplementation may be effective to improve performance and final carcass characteristics of Nellore bulls on tropical pasture only during finish phase.

Key words: corn, ruminant, soybean hulls, tropical grass

1. INTRODUCTION

Tropical grasses such as Brachiaria (*Brachiaria brizantha cv*. Xaraés) are high in NDF concentration and low in DE and CP. Therefore, supplements are often required to meet the nutrient needs of cattle that have high energy and protein requirements for the desired level of performance.

Energy supplementation in form of grain increases efficiency of energy use (NRC, 2000), greater propionate and lesser acetate production (Sutton et al., 2003), and increases the microbial production per unit of OM fermented in the rumen (Obara et al., 1991). However, high levels of starch-containing concentrates added to forage-based diets usually decrease forage intake and fiber digestion (Galloway et al., 1993; Moore et al., 1995). In contrast, soybean hulls (**SH**) with high concentrations of digestible fiber have lower starch concentrations, and feeding with SH results in less negative associative effects on forage digestion than have been observed with corn supplements (Galloway et al., 1991; Grigsby et al., 1992; Ovenell et al., 1991).

Usually, fats are fed to increase dietary energy density without reducing the fiber content (NRC, 2000), but fat supplementation has other potential benefits like improve feed efficiency, higher weight gain and deposition of body fat, promoting differences in the quantity and quality of the carcass beef cattle (Marinova et al., 2001; Oliveira et al., 2011; Ramirez and Zinn, 2000). However, high concentrations of fats may impair digestion and metabolism in the rumen, cause changes in the microbial population, and reduce feed intake of animals (Allen, 2000; Jenkins, 1993).

There have been few studies that investigated the combined effect of different carbohydrate forms and oil source on animal performance. We hypothesize that when combined with high or low-starch, fat supplementation could improve performance and carcass characteristics of young Nellore bulls maintained in tropical grass pasture. The

objective of this study was to evaluate the effects of high or low-starch supplements combined or not, with oil on performance and final carcass characteristics of young Nellore bulls grazing tropical pasture of *Brachiaria brizantha cv.* Xaraés during the growing and finish phases.

2. MATERIALS AND METHODS

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal) of the Faculty of Agriculture and Veterinary Sciences – São Paulo State University (UNESP) – Jaboticabal campus (protocol number 021119/11).

Experiment 1: Growing phase

Animals and management

The experiment was conducted at the UNESP (Jaboticabal, SP, Brazil) from December 2012 to May 2013, in the rainy season. The weather data are presented in Figure 1. Under the international Köppen classification this climate is characterized as tropical type AW with summer rains and relatively dry winter; the local altitude is 595 m, at 21°15'22" S, 48º18'58" W.

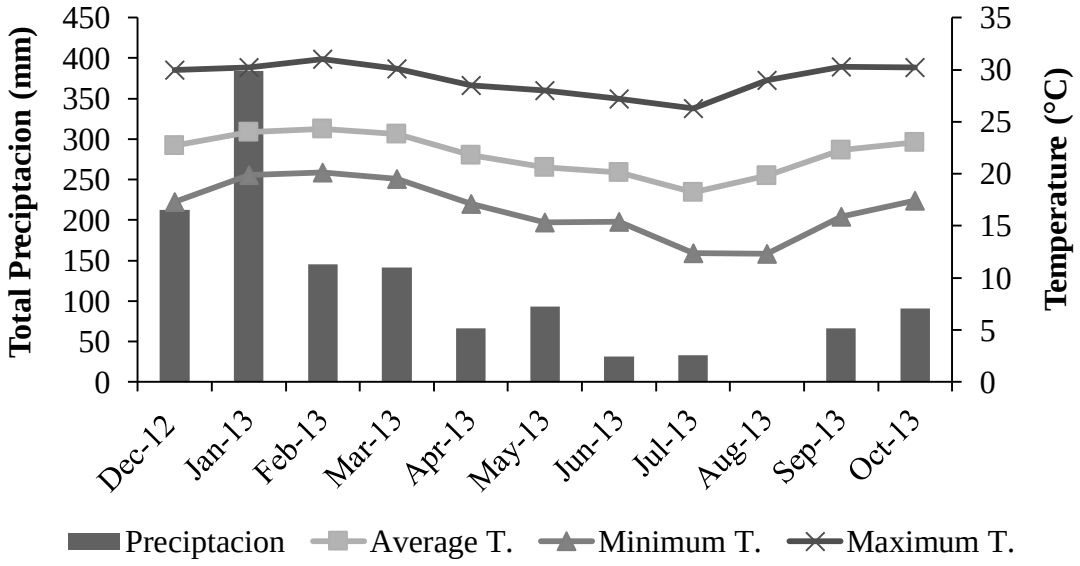

Figure 1. Precipitation, average temperature (Average T), minimum temperature (Minimum T), and maximum temperature (Maximum T) during the experimental period (January to October 2013). Rainfall days in each period: December (17), January (18), February (15), March (13), April (9), May (6), June (6), July (2), August (0), September (6), and October (8).

One hundred and four Nellore bulls were used in the experiment, with an average age of 15 mo and initial BW = 284 ± 38 kg. Carcass gain (**CrG**) was determined via the comparative slaughter technique. Eight animals (275 ± 35 kg) were slaughtered at a commercial beef plant and served as the reference group at the beginning of the experiment as the initial dressing percentage (**DP**; 50.93%), which estimated the initial carcass weight to obtain the CrG at the end of experiment. The 8 animals slaughtered were taken from a random sample.

After 133 d of feeding, another 8 animals were slaughtered at the commercial beef plant and served as the reference group at the end of the growing phase, with DP of 52.02%. The 8 animals slaughtered were taken from a random sample, with 2 animals per treatment. Carcass gain was obtained using the final estimated carcass weight (final BW × DP of final reference group) minus initial estimated carcass weight (initial BW × DP of initial reference group) per number of days feeding. Preharvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). After the slaughter, all the carcasses were weighed and refrigerated at 4°C for approximately 24 h.

The experimental period lasted 133 d, divided into an adaptation period of 21 d and 4 periods of 28 d each. Initially, the animals were weighed, identified, and treated against ecto- and endoparasites by administration of ivermectin 1% (Ivomec®; Merial, Paulínia, Brazil), and allocated into 12 paddocks of 1.8 ha, consisting of *Brachiaria brizantha* cv. Xaraés. The animals were distributed in a completely randomized design (8 animals per paddock and 3 paddocks per treatment).

The diets used consisted in *Brachiaria brizantha* cv. Xaraés pasture supplemented with two levels of starch, with or without a source of oil. The supplements were corn associated or not with ground soybean and SH associated or not with ground soybean. The proportion of ingredients and chemical composition of supplements are presented in Table 1.

Crude glycerin was used in all supplements to replace (28% of DM) corn or SH. This is a byproduct from the biodiesel agroindustry and can be used in ruminant diets without compromising intake and performance (Drouillard, 2012; Parsons et al., 2009). Crude glycerin (83.90% glycerol, 1.75% ether extract [**EE**], 4.30% ash, and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil).

Table 1. Experimental supplement and chemical composition of supplements in different phases (%DM basis)

Item	High Starch		Low Starch		High Starch		Low Starch	
	Oil	No Oil	Oil	No Oil	Oil	No Oil	Oil	No Oil
	Growing Phase				***Finishing Phase***			
Ingredient proportions								
Ground corn*	8.90	18.5	0.00	0.00	18.5	31.0	0.00	0.00
Soybean meal	0.00	49.0	0.00	49.0	0.00	38.5	0.00	37.0
Soybean hulls	0.00	0.00	8.50	18.5	0.00	0.00	18.5	32.5
Ground soybean*	58.6	0.00	59.0	0.00	51.0	0.00	51.0	0.00
Crude glycerin	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0
Commercial premix[1]	4.50	4.50	4.50	4.50	2.50	2.50	2.50	2.50
Chemical composition								
DM	90.9	88.1	90.2	88.2	90.2	89.3	90.3	89.4
CP	27.6	26.5	26.2	26.0	22.9	22.3	23.9	23.6
NDF	13.2	11.0	17.5	20.2	12.7	11.1	21.9	27.1
Starch[2]	11.0	16.3	4.79	3.52	17.2	24.7	4.45	3.29
Ether extract	13.8	3.18	13.4	2.57	12.4	3.62	11.8	2.58
GE, *Mcal/kg DM*	5.16	4.51	5.07	4.41	5.08	4.62	4.98	4.45

[1]120 g calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1.220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, 300 mg fluorine; no additive.

[2]Calculated based on ingredient values from Valadares Filho et al., 2010.

*Ground in a hammermill fitted with screen size of 3.0 mm (fine).

Animals were supplemented at the rate of 500 g/100 kg of BW, daily, at 1000 h, with 5 m of feed bunk line, and had *ad libitum* access to water and shade. The amount of supplement provided was calculated to meet the requirements for average daily gain of 1.0 kg/d, according Valadares Filho et al., 2010. Every 28 d, the animals were weighted after a 16-h period of withdrawal from feed and water, and this BW was used to adjust the amount of supplement. Average daily gain was obtained by weighing the animal at the beginning and the end of the experiment, always after a 16-h period of withdrawal from feed and water.

Grazing method used was continuous stocking with variable stocking rate ("put and take" stocking), with the use of regulator animals, with the objective of maintaining the sward height of 35 cm. Control of the stocking rate was done weekly as a function of the predetermined forage heights; that is, when the height was greater than expected for that treatment, animals were added, and in the inverse situation, animals were removed. These procedures were recorded by summing the number of grazing days for each animal in a paddock. At end of each period, the number of grazing days of all of animals in a plot were summed and divided by number of days in the period to obtain the stocking rate of each plot.

Forage height was randomly measured weekly by 80 points using a graduated stick in each paddock (Barthram, 1985). The sampling per paddock was performed every 28 d. Samples to address herbage chemical composition were obtained by hand plucking (Johnson, 1978). Every 28 d in each paddock, the average height of 80 points, using a graduated stick (Barthram, 1985), was utilized for sampling 4 sites, where all forage included within the perimeter of the rising plate (0.25 m^2) was collected by clipping at 5 cm above soil level from sites that represent the mean forage mass of paddock.

Forage mass per hectare and structural components of forage canopy were measured using the samples collected from the sites at average height and separated into green matter (stems and leaves) and dead matter (stems and leaves with more than 50% of their lengths in senescence). Subsequently, the different fractions were dried in a forced ventilation oven at 55ºC for 72 h for measurement of DM content according to AOAC (1995). The mass of each structural component was calculated as the percentage of each component (DM basis) multiplied by forage mass. Fertilizer was applied only on once during entire the experimental period, 200 kg/ha of $N:P_2O_5:K_2O$ (20:05:20), at end of rainy season (May, 2013).

Nitrogen and carbohydrate fractions

The total carbohydrates of forage were fractionated according to ruminal degradation rate. The A + B_1, B_2 and C fractions were calculated as described by Sniffen et al. (1992) as it follows: A + B_1 = 100 - (C + B_2); B_2 = 100 * (NDF (% DM) - NDIP (% CP) * 0.01* CP (% DM) - NDF (% DM) * 0.01 * LIG (% NDF) * 2.4) ÷ TCH (% DM); C = (100 * NDF (% DM) * 0.01 * LIG (% NDF) * 2.4) ÷ TCH (% DM).

The nitrogenous compounds of forage were divided into A fraction, was obtained by treatment of the sample with trichloroacetic acid (Licitra et al., 1996). The B_1 + B_2 fraction was determined by the difference between the fractions A, B_3 and C. For the difference between neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN), we determined the B_3 fraction (Sniffen et al., 1992). While the C fraction was determined by acid detergent insoluble nitrogen (Van Soest et al., 1991).

Proximate analysis

For proximate analysis, the sample of ingredients of supplements and forage were dried at 55ºC for 72 h. Samples were then ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen and analyzed for DM (method 934.01), OM (method 942.05), and EE (method 920.85) according to the Association of Official Analytical Chemists (AOAC, 1995).

Concentrations of N in each sample were determined by rapid combustion (850°C), conversion of all N-combustion products to N_2, and subsequent measurement by thermoconductivity cell (Leco model FP-528; LECO Corporation, St. Joseph, MI). Crude protein was calculated as the percentage of N in the sample multiplied by 6.25. The GE content of supplements and forage was determined using an adiabatic bomb calorimeter (model 6300; Parr Instrument Company, Moline, IL). Analyses for NDF were conducted following Van Soest et al. (1991) and adapted for the ANKOM^{200} Fiber Analyzer (Ankom Technology, Fairport, NY).

For analyses of indigestible NDF (iNDF), the samples of forage and concentrate were placed in ANKOM bags (filter bag F57; ANKOM Technology Corporation) and incubated in the rumen of a fistulated Nellore animal for a period of 288 h (Valente et al., 2011).When the bags were withdrawn from the rumen, they were soaked in water for 30 min and gently washed by hand under running water until the wash water ran clear. The bags were then

placed in an ANKOM^{200} fiber analyzer (ANKOM Technology Corporation), as described by Mertens (2002), and the iNDF was determined by weighing the bags with a digital scale after drying them in an oven, first at 55°C for 72 h and then at 105°C for 12 h. The residue was considered the iNDF.

Experiment 2: Finishing phase

The experiment was conducted at the UNESP (Jaboticabal, SP, Brazil) from May to October 2013, in the dry season. The weather data are presented in Figure 1.

Sixty Nellore bulls utilized in the growing phase were used in the finishing phase, with an average age of 20 mo and initial BW = 424 ± 34 kg. The experimental period lasted 133 d, divided into an adaptation period of 28 d and 4 periods of 28 d each. Initially, the animals were weighed, identified, and treated against ecto- and endoparasites by administration of ivermectin 1% (Ivomec®; Merial, Paulínea, Brazil), and allocated into 12 paddocks of 1.8 ha, consisting of *Brachiaria brizantha* cv. Xaraés. The animals were distributed in a completely randomized design (5 animals per paddock and 3 paddocks per treatment).

The diets used consisted of starch level, with or without a source of oil. The supplements were corn associated or not with ground soybean and SH associated or not with ground soybean. Crude glycerin, byproduct from the biodiesel agroindustry, was used in all supplements to replace (28% of DM) corn or SH. Crude glycerin (83.90% glycerol, 1.75% ether extract [**EE**], 4.30% ash, and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil). Animals were supplemented at the rate of 1000 g/100 kg of BW, daily, at 1000 h, with 3 m of feed bunk line, and had *ad libitum* access to water and shade. The proportion of ingredients and chemical composition of supplements for finish phase are presented in Table 1.

Every 28 d, the animals were weighed after a 16-h period of withdrawal from feed and water, and this BW was used to adjust the amount of supplement. Average daily gain was obtained by weighing the animal at the beginning and the end of each the experiment, always after a 16-h period of withdrawal from feed and water.

After 133 d of experiment, all the animals were slaughtered at commercial beef plant with 546.44 ± 43 kg of shrunk body weight (**SBW**). Preharvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997).

After the slaughter, the carcass was weight and all carcasses were refrigerated at 4 °C for approximately 24-h. After the postmortem chill period, 12th fat depth and 12th rib *longissimus* muscle area (**LM area**) were measured on the left side of each carcass. *Longissimus* muscle areas were traced on transparencies and measured later with a planimeter and fat depth measurements were taken 3/4 the length ventrally over the *longissimus* muscle (Greiner et al., 2003). Carcass gain was obtained using the final hot carcass weight (**HCW**) minus initial estimated carcass weight (initial BW × DP of initial reference group) per number of days feeding. Dressing percent was calculated using HCW divided by final SBW and then multiplying the result by 100. All of the feeding, weighing, and routine management were identical to that used in Exp. 1.

Statistical analysis

The experimental design was completely randomized in a 2 × 2 factorial arrangement (high or low starch, with or without a source of oil). Each paddock was considered the individual experimental unit (3 paddocks per treatment).

The mathematical model was represented by

$$Y_{ijk} = \mu + S_i + O_k + (S_i \times O_k) + e_{ijk}$$

in which Y_{ijk} = observation of paddock *j* subject to starch *i* at oil inclusion *k*, μ = the overall mean, S_i = effect of starch *i* = 1 and 2, O_k = effect of oil inclusion *k* = 1 and 2, $S_i \times O_k$ = interaction between starch *i* and oil inclusion *k*, and e_{ijk} = the residual experimental error.

The pasture characteristics and performance of animals were analyzed with starch level and oil inclusion as fixed effects and the residual error as a random effect using PROC MIXED of the SAS statistical software (SAS Inst. Inc., Cary, NC).

The chemical composition, total mass, percentage of components, carbohydrate and protein fractions of pasture, and Nellore bulls ADG were analyzed using the Mixed procedure (SAS Inst. Inc., Cary, NC) with repeated measures. The initial BW was used as a covariate for the statistical analysis of ADG, HCW, and final BW.

The data was verified for homogeneity using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Studentized residuals were plotted against the predicted values using the plot procedure to analyze data for outliers. The LSMEANS statement of the mixed procedure (SAS Inst. Inc., Cary, NC) was used to calculate mean values. When the treatments were significant, the means were compared with Fisher's tests using the PDIFF option in LSMEANS command. The level of significance used to assess differences was $\alpha = 0.05$.

3. RESULTS

In relation to chemical composition of *Brachiaria brizantha* cv. Xaraés, there were no main effects of starch level or oil, or interactions between starch × oil for OM (P = 0.59), NDF (P = 0.94), iNDF (P = 0.15), CP (P = 0.98), GE (P = 0.97), and EE (P = 0.59) among different paddocks during growing phase (rainy season). Similarly, there were no interactions between starch level and oil supplementation for OM (P = 0.29), NDF (P = 0.92), iNDF (P = 0.13), CP (P = 0.68), GE (P = 0.07), and EE (P = 0.61) of pasture during finishing phase (dry season; Table 2).

Table 2. Chemical composition of the *Brachiaria brizantha* cv. Xaraés during different phases (% DM basis)

Item	High Starch		Low Starch			*P-value*			
	Oil	No Oil	Oil	No Oil	SEM	Time	Starch	Oil	Starch × Oil
Growing Phase[1] *(December 2012 to May 2013)*									
OM	92.48	92.46	92.37	92.57	0.19	< 0.01	0.98	0.65	0.59
NDF	56.18	56.16	55.47	55.58	1.03	0.02	0.54	0.96	0.94
iNDF	14.22	14.60	14.65	13.50	0.48	< 0.01	0.50	0.45	0.15
CP	12.51	12.92	12.70	13.13	0.51	< 0.01	0.71	0.44	0.98
GE	4.45	4.46	4.44	4.45	8.50	< 0.01	0.87	0.83	0.97
EE	1.35	1.34	1.37	1.20	0.14	< 0.01	0.67	0.53	0.59
Finishing Phase[2] *(May to October 2013)*									
OM	92.68	92.79	92.84	92.73	0.09	0.67	0.59	0.98	0.29
NDF	52.32	52.63	51.92	52.37	0.67	< 0.01	0.64	0.59	0.92
iNDF	13.06	14.04	13.13	13.02	0.32	< 0.01	0.18	0.21	0.13
CP	12.94	13.17	13.44	13.20	0.55	< 0.01	0.64	0.99	0.68
GE	4.56	4.55	4.55	4.56	2.37	< 0.01	0.54	0.45	0.07
EE	2.91	3.22	3.13	3.22	0.20	< 0.01	0.61	0.36	0.61

[1]Growing phase (High starch: 136 g/kg of starch in DM supplement; Low starch: 41.5 g/kg of starch in DM supplement).
[2]Finish phase (High starch: 209 g/kg of starch in DM supplement; Low starch: 38 g/kg of starch in DM supplement).

However, there was effect of evaluation time ($P < 0.01$) on chemical composition of pasture at total experimental period. In the final experimental period, the reduction in height was due to climatic conditions, which favored the reduction of pasture growth rates (Figure 2).

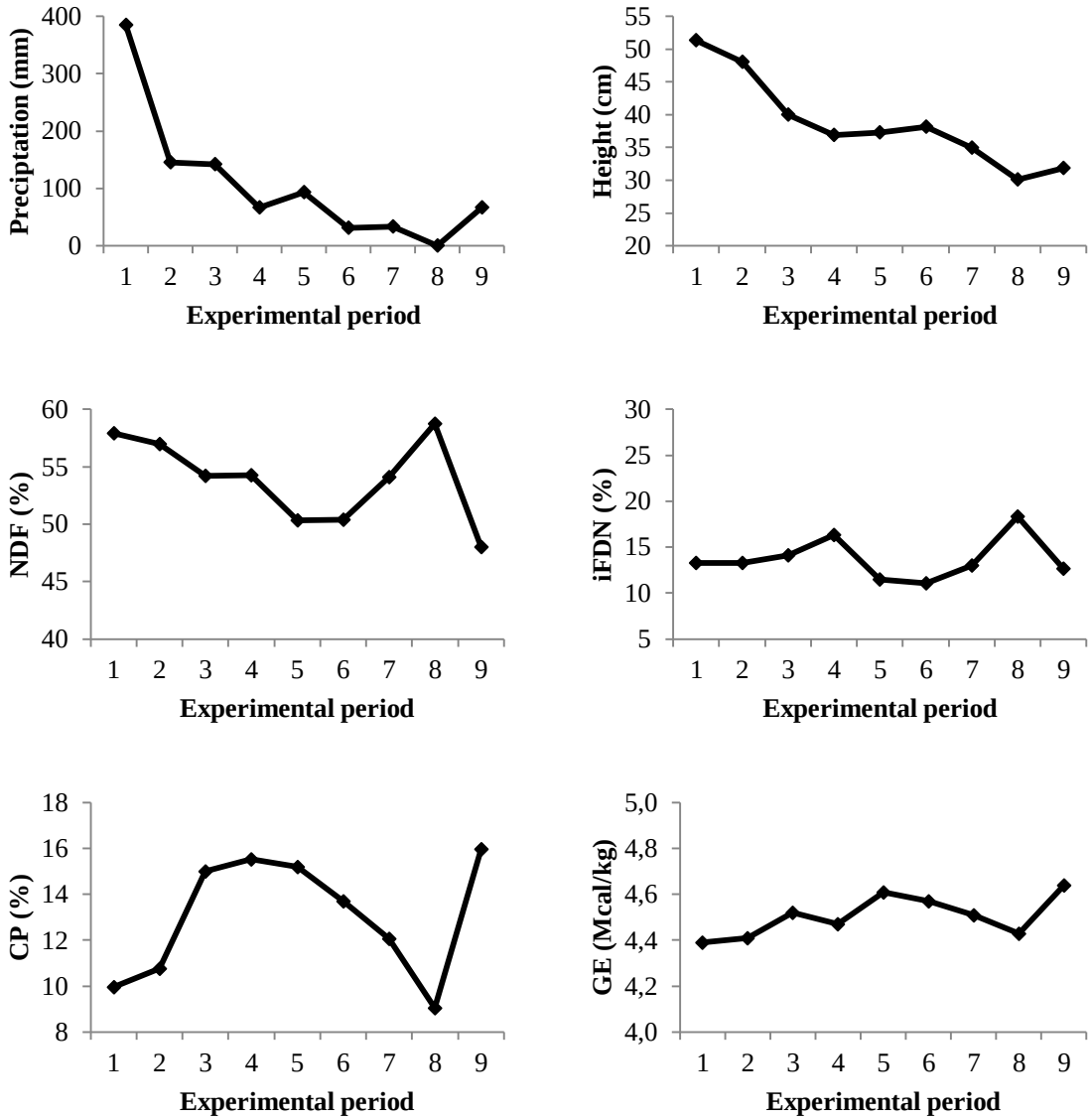

Figure 2 – Total precipitation (mm), height (cm), neutral detergent fiber (NDF), indigestible neutral detergent fiber (iNDF), crude protein (CP) and gross energy (GE) of the *Brachiaria brizantha cv*. Xaraés during different experimental period (December 2012 to October 2013). The forage samples were obtained by hand plucking (Johnson, 1978).

There were no main effects of starch level or oil, or interaction between starch × oil for total herbage mass (P = 0.83), dead mass (P = 0.61), leaf mass (P = 0.24), stem mass (P = 0.86), leaf/stem ratio (P = 0.35), height (P = 0.40), and stocking rate (P = 0.42) of pasture evaluated during growing phase. In relation to finishing phase, there were no interactions between starch level and oil supplementation for total herbage mass (P = 0.09), dead mass (P = 0.63), leaf mass (P = 0.92), stem mass (P = 0.51), leaf/stem ratio (P = 0.75), height (P = 0.14), and stocking rate (P = 0.25) of pasture among different paddocks (Table 3).

Table 3. Total herbage mass, percentage and mass of the components in *Brachiaria brizantha* cv. Xaraés pastures under a continuous stocking system during different phases

Item	High Starch		Low Starch		SEM	*P-value*			
	Oil	No Oil	Oil	No Oil		Time	Starch	Oil	Starch × Oil
Growing Phase									
Forage mass, kg/ha	10535.00	9498.91	9478.61	8656.92	509.05	0.04	0.09	0.10	0.83
Dead mass, %	32.09	38.89	35.09	38.42	3.28	< 0.01	0.70	0.16	0.61
Leaf mass, %	26.67	23.45	25.43	24.89	1.06	< 0.01	0.92	0.11	0.24
Stem mass, %	41.24	37.66	39.48	36.69	2.32	0.01	0.57	0.20	0.86
Leaf/Stem ratio	0.62	0.61	0.64	0.67	0.02	< 0.01	0.14	0.67	0.35
Height, cm	46.31	43.05	44.12	42.72	1.05	< 0.01	0.26	0.05	0.40
Stocking rate, AU/ha	4.64	4.38	4.71	4.15	0.18	< 0.01	0.67	0.05	0.42
Finishing Phase									
Forage mass, kg/ha	9211.07	7941.24	8085.33	8370.38	441.24	0.01	0.41	0.26	0.09
Dead mass, %	70.98	69.13	70.24	71.10	2.89	< 0.01	0.83	0.86	0.63
Leaf mass, %	15.22	15.08	14.67	14.77	1.32	< 0.01	0.73	0.98	0.92
Stem mass, %	13.80	15.79	15.09	14.13	2.33	< 0.01	0.91	0.80	0.51
Leaf/Stem ratio	1.13	1.08	1.10	1.15	0.16	< 0.01	0.88	0.98	0.75
Height, cm	36.89	33.35	32.84	34.75	1.69	< 0.01	0.45	0.64	0.14
Stocking rate, AU/ha	2.93	3.01	3.34	3.00	0.17	< 0.01	0.28	0.46	0.25

[1]Growing phase (High starch: 136 g/kg of starch in DM supplement; Low starch: 41.5 g/kg of starch in DM supplement).

[2]Finish phase (High starch: 209 g/kg of starch in DM supplement; Low starch: 38 g/kg of starch in DM supplement).

However, there was effect of evaluation time ($P < 0.01$) on values of percentage of structural components, height and stocking rate of forage during period experimental (Table 3). In the growing phase, there were no main effects of starch level or oil, or interaction between starch × oil for fractions of carbohydrate A + B_1 ($P = 0.77$), B_2 ($P = 0.83$) and C ($P = 0.57$), and for fractions of protein A ($P = 0.71$), $B_1 + B_2$ ($P = 0.58$), B_3 ($P = 0.37$) and C ($P = 0.38$) of *Brachiaria brizantha* cv. Xaraés.

Table 4. Values of carbohydrate and protein fractions of the *Brachiaria brizantha* cv. Xaraés during different season (% DM basis)

Item	High Starch		Low Starch		SEM	*P-value*			
	Oil	No Oil	Oil	No Oil		Time	Starch	Oil	Starch × Oil
Growing Phase									
Carbohydrate									
A + B_1	11.39	10.40	11.68	11.63	1.57	< 0.01	0.64	0.75	0.77
B_2	81.87	82.62	81.63	81.84	1.22	< 0.01	0.68	0.70	0.83
C	6.74	6.98	6.69	6.53	0.51	< 0.01	0.51	0.91	0.57
Protein									
A	30.29	24.11	29.76	27.50	4.95	< 0.01	0.78	0.44	0.71
$B_1 + B_2$	35.48	43.31	38.19	38.73	6.18	< 0.01	0.88	0.53	0.58
B_3	30.88	29.11	28.48	30.18	1.73	0.01	0.72	0.98	0.37
C	3.35	3.47	3.57	3.59	0.17	< 0.01	0.75	0.79	0.38
Finishing Phase									
Carbohydrate									
A + B_1	13.35	12.29	13.23	12.59	1.04	< 0.01	0.93	0.43	0.84
B_2	78.69	79.43	78.39	78.85	0.80	< 0.01	0.59	0.47	0.86
C	7.96	8.28	8.38	8.56	0.42	< 0.01	0.42	0.56	0.87
Protein									
A	25.90	28.14	28.46	25.86	1.24	< 0.01	0.91	0.89	0.12
$B_1 + B_2$	45.09	44.97	40.65	44.75	2.05	0.13	0.32	0.38	0.36
B_3	26.03	23.69	27.60	26.27	2.17	0.75	0.39	0.44	0.82
C	2.98	3.20	3.29	3.12	0.12	< 0.01	0.17	0.77	0.36

[1]Growing phase (High starch: 136 g/kg of starch in DM supplement; Low starch: 41.5 g/kg of starch in DM supplement).

[2]Finish phase (High starch: 209 g/kg of starch in DM supplement; Low starch: 38 g/kg of starch in DM supplement).

In addition, there were no interactions between starch level and oil supplementation for fractions of carbohydrate A + B_1 (P = 0.84), B_2 (P = 0.86) and C (P = 0.87), and for fractions of protein A (P = 0.12), B_1 + B_2 (P = 0.36), B_3 (P = 0.82) and C (P = 0.36) of forage during finishing phase. However, there was effect of evaluation time (P < 0.01) on values of carbohydrate and protein fractions of *Brachiaria brizantha* cv. Xaraés during different season (Table 4).

Table 5. Effects of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on initial and final BW, ADG, total gain (TG) and carcass gain (CrG) of Nellore bulls on pasture during different phase

Item	High Starch		Low Starch		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
***Growing Phase*[1]**								
Initial BW, kg	274.29	289.00	290.58	281.88	7.86	0.56	0.70	0.14
Final BW, kg	384.60	398.18	390.62	394.97	3.73	0.70	0.01	0.22
ADG, kg/d	0.94	1.03	0.92	1.01	0.02	0.36	< 0.01	0.20
Total Gain, kg	103.75	113.97	101.99	111.28	2.96	0.44	< 0.01	0.87
CrG, kg/d	0.50	0.53	0.53	0.57	0.01	< 0.01	0.01	0.86
***Finishing Phase*[2]**								
Initial BW, kg	411.07	432.33	422.71	428.27	9.26	0.67	0.14	0.38
Final BW, kg	556.76	539.92	546.65	542.44	4.06	0.34	0.01	0.11
ADG, kg/d	0.91	0.80	0.85	0.83	0.02	0.85	0.02	0.11
Total Gain, kg	133.15	116.31	123.04	118.83	4.06	0.34	0.01	0.11
HCW, kg	321.25	311.18	320.29	314.53	2.79	0.66	< 0.01	0.43
Dressing, %	57.58	57.72	58.54	58.00	0.34	0.07	0.55	0.31
CrG, kg/d	0.73	0.67	0.68	0.64	0.01	0.07	0.01	0.56
Fat depth, mm	4.51	3.76	4.21	3.66	0.31	0.52	0.04	0.74
LM area, cm^2	81.33	85.67	83.57	86.11	2.93	0.64	0.23	0.75

[1]Growing phase (High starch: 136 g/kg of starch in DM supplement; Low starch: 41.5 g/kg of starch in DM supplement).
[2]Finish phase (High starch: 209 g/kg of starch in DM supplement; Low starch: 38 g/kg of starch in DM supplement).

In relation to animal performance during growing phase, there were no interaction between starch × oil for final BW ($P = 0.22$), ADG ($P = 0.20$), total gain ($P = 0.87$), and CrG ($P = 0.86$). However, the addition of oil decreased final BW ($P = 0.01$), ADG ($P < 0.01$), total gain ($P < 0.01$) and CG ($P = 0.01$). In addition, animals supplemented with high starch decreased CrG ($P < 0.01$; Table 5).

On the other hand, during finishing phase, there were no interactions between starch level and oil for initial BW ($P = 0.38$), final BW ($P = 0.11$), ADG ($P = 0.11$), total gain ($P = 0.11$), HCW ($P = 0.43$), dressing ($P = 0.31$), CrG ($P = 0.56$), fat depth ($P = 0.74$), and LM area ($P = 0.75$). In contrast, animals supplemented with oil increased final BW ($P = 0.01$), ADG ($P = 0.02$), total gain ($P = 0.01$), HCW ($P < 0.01$), CrG ($P = 0.01$), and fat depth ($P = 0.04$). Furthermore, there was effect of time during growing and finishing phase on values of ADG ($P < 0.01$) of Nellore bulls fed tropical pasture (Table 5).

4. DISCUSSION

In this study evaluated the effects of high- or low-starch supplements combined or not, with oil on performance and final carcass characteristics of young Nellore bulls fed tropical pasture during the growing and finish phase. The addition of oil source to the diet was associated with reduced on animal performance in growing phase. However, animals supplemented with oil source increased performance during the finishing phase.

Overall, the forage offered for animals among the treatments during growing (rainy season) and finishing phase (dry season) showed the same nutritional quality, as exemplified by no differences for OM, NDF, iNDF, CP, GE e EE (Table 2). However, chemical composition only varied as a function of the experimental period. Advancing maturity generally increases the indigestible fraction and decreases rate of digestion of forage NDF (Smith et al., 1972), which will have counteracting effects on the length of time a particle is buoyant (Jung and Allen, 1995).

Total forage mass, percentage of the structural components, leaf/stem ratio, height and stocking rate of forage were similar among all paddocks. This was expected, since that the pasture management criteria used was the same in all combinations of treatments. This outcome most likely reflects the same proportion of leaf, accumulation of stems and dead material (Hoogendoorn et al., 1992; Wims et al., 2010). In the final experimental period (dry

season), the reduction in height was due to climatic conditions, which reduced the growth rates (Figure 2).

The purpose of evaluating the carbohydrates and proteins fractions is the maximization of the efficiency of growth of the ruminal microorganisms (Russell et al., 1992). These are categorized as bacteria that ferment fiber carbohydrate (FC) and non-fiber carbohydrate (NFC) (Russell et al., 1992; NRC, 2000). The FC bacteria degrade cellulose and hemicellulose, grow more slowly, and utilize ammonia as their primary N source for microbial protein synthesis. The NFC bacteria utilize starch, pectin, and sugars and grow more rapidly than FC bacteria and can utilize ammonia or AA's as N sources. The rate of NFC and FC bacterial growth is controlled by the amount of carbohydrate that is digested in the rumen and the rate of carbohydrate digestion (*Kd*) so long as adequate N sources and other essential nutrients are available (Tylutki et al., 2008).

The non-structural carbohydrates (A + B_1 fraction) are sources of quickly available energy. The A fraction is a very rapidly fermented, water soluble, pool that is largely composed of sugars, although it also contains organic acids and short oligosaccharides. The B_1 fraction, with a slower digestion rate (*Kd*) than A fraction, is primarily starch and pectin. The B_2 pool is composed of available and potentially degradable NDF (Sniffen et al., 1992). The increase in the proportion of the B_2 fraction of carbohydrates is correlated with the level of NDF in the forage that supplies energy in a relatively slow rate may affect the efficiency of microbial synthesis and animal performance (Russell, 1998). The C fraction corresponds to the non-digestible percentage of the neutral detergent fiber (Sniffen et al., 1992). The increase of this fraction results in a larger effect in the ruminal repletion and a decrease of the energy availability due to indigestibility along the digestive tract (Jung and Allen, 1995).

Protein A fraction of CP is non-protein nitrogen (NPN) that enters the ruminal ammonia pool directly. B_1 fraction is true protein that has a rapid *Kd* and is nearly completely degraded in the rumen. The B_2 fraction is partly degraded in the rumen, although forages rich in the fraction B_2 require NPN to attend to nitrogen requirements of rumen microorganisms which ferment structural carbohydrates (Russell et al., 1992). The B_3 fraction or slowly degraded protein fraction is determined by subtracting the value of acid detergent insoluble protein (ADIP) from the value determined for neutral detergent insoluble protein (NDIP). The C fraction is ADIP and is assumed unavailable.

The high proportions of protein A and B_1 fractions, with their high digestion rates, can cause larger losses of ammonia, when sources of fast degradation carbohydrates are not available in the rumen. It requires a good synchronism in the fermentation of proteins and carbohydrates, for efficient microbial synthesis in the rumen and consequent improvement in the animal performance (Nocek and Russel, 1998). Thus, when the A + B_1 fraction constitutes the main fraction of carbohydrates in the diet, it is necessary to include protein sources of fast and intermediate degradability in the rumen for the synchronization between the energy and the nitrogen releases (Cabral et al., 2000).

In general, the levels of the carbohydrates fractions and nitrogenous compounds of the foods together with the parameters of kinetics of ruminal degradation supply more adequate information on the nutritional value of the foods than only the chemical composition values (Campos et al., 2010). Thus, interactions between supplementary dietetic fractions and basal diet (pasture) determine ruminal (degradation rate and passage rate) and metabolic (animal physiological control) effects on intake and utilization of feed, with different effects from the same supplement in different seasons.

In relation to the performance characteristics, no differences were observed for interaction between starch level and oil supplementation during growing and finishing phase. However, for animals fed with oil was observed effect unlike between the phases. In this study, the largest supplement intake in the finishing phase, consequently of oil and energy, increased the ADG and improved animal carcass characteristics in relation to the growing phase.

Dietary fat plays an important role in provision of energy to the animal and the energy concentration of the fatty acid (FA) source is primarily determined by digestibility of the fat. On average, the digestibility of saturated fatty acids increases moderately with chain length and that unsaturated fatty acids are more digestible than saturated fatty acids (Doreau and Ferlay, 1994; Doreau and Chilliard, 1997).This digestibility is affected by physical nature of the fat source, whether the fat source is a triglyceride or free FA, level of DM and FA intake, and FA composition of the fat source (Hall and Eastridge, 2014).

Lipid metabolism begins with the dietary intake of feedstuffs, which is followed by an extensive ruminal de-esterification and biohydrogenation of dietary lipids as well as the formation of short-chain fatty acids from dietary fiber compounds and fermentable carbohydrates, an absorption of fatty acids and fatty acid precursors, and further

digestion/absorption processes of ruminally unaffected/protected lipids along the intestine (Bauman et al., 2003; Lock et al., 2006; Scollan et al., 2014). Upon absorption, fatty acids and fatty acid precursors are transported to target tissues for further metabolisation, syntheses, deposition and/or excretion (Dodson et al., 2010; Hocquette and Bauchart, 1999).

The biohydrogenation process in the rumen is affected by many factors such as feed intake, diet composition, the type and source of carbohydrates, the degree of fatty acid unsaturation, the forage to grain ratio and the nitrogen content of the diet (Chouinard et al., 2001; Piperova et al., 2000; Song and Kenelly, 2003).

When the fiber content of the diet is lower, and higher levels of concentrates are used in the diet, there is a reduction in the number of cellulolytic bacteria in the rumen (Doreau and Ferlay, 1994; Loor et al., 2004; Kalscheur et al., 1997). Thus this kind of diet favors lipids which pass the rumen without being reduced, especially unsaturated fatty acids (Chilliard et al., 2007).

Variation in the dietary energy content or fat content had pronounced effects on the amount of free FA stored in lipid droplets in bacteria (Bauchart et al., 1990). These results suggest that a higher diet energy density increase the yield of energy stored by ruminal microorganisms as FA, in the same manner that it increases the concentration of bacteria in the rumen (Dehority and Orpin, 1988).

Fat deposition can be characterized chemically by continual accretion of primarily triacylglycerol, and morphologically by adipocyte differentiation and hypertrophy. The level of food intake and the composition of food regulate the rate of fatty tissue growth and the composition of lipids (Nürnberg et al., 1998). Carcass composition can be modified by altering the energy intake (Ferreira et al., 1998).

The implications of this study are the nutritional factors to be considered in the productive systems of grazing animals. In addition to the correct management of tropical forages, the use of concentrate supplementation is a technology that enables greater system efficiency, during the rainy and dry season, enabling an increase in pasture stocking rate, supporting additional gains and, consequently, increase gain per area. Based on these changes, the production of the Brazilian beef cattle can improve their efficiency and contribute to global food production. Such as stocking rate of the 0.54 UA/ha (IBGE, 2001) to results found in this study of 3.77 UA/ha.

5. CONCLUSION

High or low-starch supplements have a same feeding value to Nellore bulls on tropical pasture during growing and finish phase. Animals supplemented with low-starch incresead carcass gain in the growing phase. The use of oil source supplementation may be effective to improve performance and final carcass characteristics of Nellore bulls on pasture of *Brachiaria brizantha cv.* Xaraés only during finish phase.

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CHAPTER 3

O artigo a seguir está publicado e redigido conforme normas do *Journal of Animal Science*, exceto o posicionamento das tabelas e figuras.

EFFECT OF STARCH-BASED SUPPLEMENTATION LEVEL COMBINED WITH OIL ON INTAKE, PERFORMANCE, AND METHANE EMISSIONS OF GROWING NELLORE BULLS ON PASTURE

ABSTRACT: Intake of tropical grass forages alone is generally insufficient to avoid nutrition imbalances and reduced animal performance, thus supplementation is often recommended. The hypothesis of the present study is that when combined with fat, soybean hulls (SH) could replace corn as a source of energy, reducing methane production without affecting animal performance. This study evaluated the effects of starch-based supplementation level combined with oil on intake, digestibility, performance, and methane emissions of growing young Nellore bulls (n = 44, initial BW = 250.69 ± 27 kg) fed *Brachiaria brizantha* cv. Xaraés during the rainy season. There were no interactions between starch level and oil supplementation with regard to intake of DM (P = 0.67), forage DM (P = 0.55), supplement DM (P = 0.14), OM (P = 0.66), CP (P = 0.74), NDF (P = 0.50), EE (P = 0.47) and GE (P = 0.68). The intake of EE was greater for animals supplemented with oil than those fed supplements without oil (P < 0.01). There were no interactions between starch level and oil supplementation on digestibility of DM (P = 0.18), OM (P = 0.11), NDF (P = 0.42), and EE (P = 0.14). Moreover, there was interaction between starch and oil supplementation on GE (P < 0.01). Independently of starch level utilized, the addition of oil decreased the digestibility of OM (P = 0.04) and NDF (P = 0.03). There were no main effects of starch level or oil, or interaction between starch × oil for initial BW (P = 0.10), final BW (P = 0.94), ADG (P = 0.40), FE (P = 0.37), and CG (P = 0.38). There was no interaction between starch-based supplementation level and oil on methane emissions when expressed in g/d (P = 0.77), kg/yr (P = 0.77), g/kg DMI (P = 0.53), and g/kg CG (P = 0.31). There was, however, an interaction (P = 0.04) between starch level and oil on methane emissions when corrected for NDF intake. Additionally, oil decreased enteric methane emission for intake of GE (P = 0.04) and EE (P < 0.01) of animals fed with starch level. Soybean hulls have the similar estimated feeding value to that of corn. The use of oil supplementation may be effective to reduce enteric methane emission of Nellore bulls raised on pasture.

Key words: corn, greenhouse gases, ruminant, soybean hulls, tropical grass

1. INTRODUCTION

Feeding systems based exclusively on tropical grass may compromise optimal cattle growth. Even though the nutritive value of grasses is greater in the rainy season, as indicated by increased CP content, nutrient imbalances are still present (Detmann et al., 2008). Indeed, nitrogen utilization in grazing beef cattle is often low due to high concentrations of rapidly soluble and degradable protein in pasture-based diets if energy is not simultaneously available. Under these circumstances, supplementation with energy is often recommended (Higgs et al., 2013).

Supplements may affect ruminal fermentation in different ways, depending on the chemical forms of carbohydrates in their composition (Costa et al., 2009). When starch-based supplements are provided in conjunction with forage-based diets, DMI and fiber digestion are often reduced, and so are methane emissions (Moss et al., 2000). Soybean hulls, in turn, have been shown to be an energy source comparable to corn (Santana et al., 2013), but their effect on fiber digestion is not as intensive (Ludden et al., 1995).

The addition of fats to ruminant diets has also been recommended, as it similarly increases energy efficiency and hence reduces methanogenesis. Although greater concentrations of fats decrease methane production substantially, they often exert detrimental effects on fiber digestion, and consequently animal performance (Patra, 2013).

There have been few studies to date that investigated the combined effect of different carbohydrate forms and oil sources on animal performance and methane emission. The hypothesis of the present study is that when combined with fat, SH could replace corn as a source of energy, reducing methane production without affecting performance. This study evaluated the combined effects of high- or low-starch supplements and oil on intake, digestibility, performance, and methane emissions of young Nellore bulls fed *Brachiaria brizantha* cv. Xaraés during the rainy season.

2. MATERIALS AND METHODS

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation (COBEA – Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA – Comissão de Ética e Bem Estar Animal) of the FCAV–UNESP–Jaboticabal campus (protocol number 021119/11).

Animals and management

The experiment was conducted at the São Paulo State University (UNESP, Jaboticabal, SP, Brazil) from December 2012 to May 2013, in the rainy season. Under the international Köppen classification this climate is characterized as tropical type AW with summer rains and relatively dry winter; the local altitude is 595 m, at 21°15'22" south latitude, 48º18'58" west longitude.

Forty-four Nellore bulls were used in the experiment, with an average age of 15 mo and initial body weight (IBW) = 250.69 ± 27 kg. Carcass gain (CrG) was determined via the comparative slaughter technique. Eight animals (275.16 ± 35 kg) were slaughtered at a commercial beef plant and served as the reference group at the beginning of the experiment as the initial dressing percentage (DP) (50.93%), which estimated the initial carcass weight to obtain the CrG at the end of experiment. The eight animals slaughtered were taken from a random sample.

After 133 d of feeding, again eight animals were slaughtered at the commercial beef plant and served as the reference group at the end of the experiment, with DP of 52.02%. The eight animals slaughtered were taken from a random sample, with two animals per treatment. For obtain the CrG was used the estimate the carcass weight initial (CWi = BWi × DP of reference group) minus the estimate the carcass weight final (CWf = BWf × DP of reference group) per number of days feeding. Pre-harvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2004). After the slaughter, all the carcasses were weighed and refrigerated at 4°C for approximately 24 h.

The experimental period lasted 133 d, divided into an adaptation period of 21 d and four periods of 28 d each. Initially, the animals were weighed, identified, and treated against ecto- and endoparasites by administration of ivermectin 1% (Ivomec®, Merial, Paulínea, BR), and allocated into 12 paddocks of 1.8 ha, consisting of *Brachiaria brizantha* cv. Xaraés. The animals were distributed in a completely randomized design (three animals per paddock and three paddocks per treatment).

The diets used consisted of starch level, with or without a source of oil. The supplements were corn associated or not with ground soybean, and SH associated or not with ground soybean. Crude glycerin is a byproduct from the biodiesel agroindustry and can be used in ruminant diets without compromising intake and performance (Drouillard, 2012;

Parsons et al., 2009). This byproduct was used in all supplements to replace (28% of DM) corn or SH. Crude glycerin (83.90% glycerol, 1.75% ether extract, 4.30% ash, and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (CARGILL, Três Lagoas, Mato Grosso do Sul, Brazil). The proportion of ingredients and chemical composition of supplements are presented in Table 1.

Table 1. Experimental supplement and chemical composition of supplements and pasture (% DM basis)

Item	High Starch		Low Starch		Pasture[1]
	Oil	No Oil	Oil	No Oil	
Ingredient proportions					
Ground corn[*]	8.90	18.5	0.00	0.00	-
Soybean meal	0.00	49.0	0.00	49.0	-
Soybean hulls	0.00	0.00	8.50	18.5	-
Ground soybean[*]	58.6	0.00	59.0	0.00	-
Crude glycerin	28.0	28.0	28.0	28.0	-
Commercial premix[2]	4.50	4.50	4.50	4.50	-
Chemical composition					
Dry matter	90.9	88.1	90.2	88.2	-
Crude protein	27.6	26.5	26.2	26.0	12.8
NDF	13.2	11.0	17.5	20.2	59.0
Starch[3]	11.0	16.3	4.79	3.52	-
Ether extract	13.8	3.18	13.4	2.57	1.32
Gross energy, *Mcal/kg DM*	5.16	4.51	5.07	4.41	4.45

[1]Average of samples obtained by technique of simulated grazing in 5 periods.

[2]120 g calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1.220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, 300 mg fluorine; no additive.

[3]Calculated based on ingredient values from Valadares Filho et al., 2010.

[*]Ground in a hammermill fitted with screen size of 3.0 mm (fine).

Animals were supplemented at the rate of 500 g/100 kg of BW, daily, at 1000 h and had ad libitum access to water and shade. Every 28 d the animals were weighed, after a 16 h withdrawal period from feed and water, and this BW was used to adjust the amount of

supplement. Average daily gain (ADG) was obtained by weighing the animal at the beginning and the end of the experiment, always after a 16 h withdrawal period from feed and water.

Grazing method used was continuous stocking with variable stocking rate ("put and take" stocking), with the use of regulator animals, with the objective of maintaining the sward height of 35 cm. Control of the stocking rate was done weekly as a function of the predetermined forage heights, i.e., when the height was greater than expected for that treatment, animals were added, and in the inverse situation, animals were removed.

Forage height was randomly measured weekly by eighty points using a graduated stick, in each paddock (Barthram, 1985). Samples to address herbage chemical composition were obtained by hand plucking (Johnson, 1978). Hand plucking was performed on the same days as the estimation of DMI, described later. The simulation of grazing per paddock was performed every 28 d.

Proximate analysis

For proximate analysis, the sample of ingredients of supplements, forage, and feces were dried at 55ºC for 72 h. Samples were then ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen, and analyzed for dry matter (DM, method 934.01), organic matter (OM, method 942.05), and ether extract (EE, method 920.85) in accordance with AOAC (1995). Concentrations of nitrogen (N) in each sample were determined by rapid combustion (850°C), conversion of all N-combustion products to N_2, and subsequent measurement by thermoconductivity cell Leco®, model FP-528 (LECO Corporation, Michigan, USA). Crude protein was calculated as the percentage of N in the sample multiplied by 6.25. The gross energy content of supplements, forage, and feces was determined using an adiabatic bomb calorimeter (PARR Instrument Company 6300, Moline, IL, USA). Analyses for neutral detergent fiber were conducted using the batch procedures outlined by ANKOM Technology Corporation (Fairport, NY). Heat-stable α-amylase was included in the NDF solution, without added sodium sulfite.

Intake estimation

Thirty-six animals were used to estimate intake and nutrient digestibility. LIPE® (lignin isolated, purified, and enriched from *Eucalyptus grandis*) and indigestible neutral

detergent fiber (iNDF) were used to estimate the excretion of fecal matter (as dry weight) and forage intake, respectively.

LIPE® was provided for 7 d by oral administration of a 500 mg bolus, with 4 d to stabilize fecal excretion of the marker, and in the last 3 d for fecal sample collection (Saliba, 2005). Fecal samples were collected during 3 d, directly from the rectum, at 1600, 1100 and 0700 h, on the first, second and third day of collection, respectively. The fecal samples were dried at 55°C for 72 h and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen, and composited proportionately on each of 3 d of sampling, within each animal, based on fecal dry weights. Approximately 10 g of each composited sample of feces was sent to the Federal University of Minas Gerais to estimate the total daily fecal output by two methods of LIPE® measurement as described by Saliba (2005). Individual concentrate intake was estimated by dividing the total concentrate provided by the number of animals in each paddock.

The individual intakes of forage were estimated using the internal marker iNDF. The samples of feces, forage, and concentrate were placed in ANKOM bags (Filter bag F57) and incubated in the rumen of a fistulated Nellore animal for a period of 288 h (Valente et al., 2011). When the bags were withdrawn from the rumen, they were soaked in water for 30 min and gently washed by hand under running water until the wash water ran clear. The bags were then placed in an ANKOM 200 fiber Analyzer, as described by Mertens (2002), and the iNDF was determined by weighing the bags with a digital scale after drying them in an oven, first at 55°C for 72 h followed by 105°C for 12 h. The residue was considered the iNDF. Individual forage intakes were estimated by subtracting marker excretion from the concentrate from the total iNDF excretion and dividing that difference by the concentration of the marker in the forage.

Methane measurements

The methane emissions were assessed using the sulfur hexafluoride (SF_6) tracer technique (Johnson et al., 1994), where each animal was sampled daily for six consecutive 24 h days, beginning on d 65 of feeding. Thirty-six animals were fitted with gas collection halters at 14 d before methane sampling to allow animals to adapt and facilitate sampling.

The release rate (RR) of the gas from a permeation tube is known before its insertion into the rumen. The permeation tubes were maintained in a water bath at 39°C and weighed in

the laboratory for 7 wk. The average RR was similar among the treatments (RR 1.90 ± 0.2 mg SF_6/d, mean ± SD). Brass permeation tubes filled with SF_6 and known release rates were administered orally to each of the thirty-six animals 72 h before methane sampling to allow the tracer gas to equilibrate in the rumen. The animals were fitted with gas collection halters connected to preevacuated polyvinyl chloride (PVC) canisters designed to fill halfway over 24 h. Eructated gas sampling started at 0700 h daily, when the animals were removed from the paddocks, and was conducted at the management center (stockyard) to facilitate sampling.

The collection canisters were located above each animal to reduce the risk of equipment damage and were connected to the halter by tubing inside airline flexible-coil tubing. Collection canisters, constructed of PVC pipe, were attached to a vacuum pump in the laboratory to create a negative pressure. As the vacuum in the sampling canister was slowly dissipated, the negative pressure steadily drew the sample of air from around the mouth and nose of the animal. The pressure of the canister, removed from the animal, was measured after 24 h of collection and if the final pressure was out of the expected range, the halter was preventively replaced. If the final pressure was above the expected range, the halter was probably blocked or disconnected; if the final pressure was below the expected range, possibly there was a leak in the system. In both situations a new halter was placed on the animal with an average absorption rate within the stipulated range (fill halfway over 24 h).

After sampling (approximately 30 min), each animal was brought to the original paddock for feeding. The pressure readings were recorded, and the canisters were pressurized using pure N_2. Additional canisters were placed near the experimental pasture to monitor background levels of methane and SF_6 daily during each sampling period. These values were subtracted from the animals' values to calculate the net output in the expired breath. The concentrations of CH_4 and SF_6 in the collection tubes were measured at the Laboratory of Animal Nutrition – UNESP (Jaboticabal, SP, Brazil) using a Shimadzu CG-2014 ATF model gas chromatograph (Agilent, San Jose, CA, USA), capillary column Porapak Q, FID detector for methane, and ECD detector for hexafluoride, as described by Johnson et al. (1994).

CH_4 flux produced by animals was calculated in relation to the SF_6 tracer gas flux from a permeation capsule lodged in the rumen minus the basal CH_4 concentration in the air (Westberg et al., 1998).

The following equation was used:

$$Q_{CH4} = Q_{SF6} \times ([CH_4]_y - [CH_4]_b) \cdot [SF_6]^{-1}$$

Where Q_{CH4} = CH_4 emission tax by animal; Q_{SF6} = know SF_6 emission tax from capsule in rumen; $[CH_4]_y$ = CH_4 concentrations in collection apparatus; $[CH_4]_b$ = basal CH_4 concentration; and $[SF_6]$ = SF_6 concentration in collection apparatus.

Statistical analysis

The experimental design was completely randomized in a 2 × 2 factorial arrangement (high or low starch, with or without a source of oil). Each paddock was considered as the individual experimental unit (three animals per paddock and three paddocks per treatment).

The mathematical model was represented by: $Y_{ijk} = \mu + S_i + O_k + (S_i \times O_k) + e_{ijk}$

Where: Y_{ijk} = observation of paddock j subject to starch i at oil inclusion k;

μ = the overall mean;

S_i = effect of starch i = 1 and 2;

O_k = effect of oil inclusion k = 1 and 2;

$S_i \times O_k$ = interaction between starch i and oil inclusion k; and

e_{ijk} = the residual experimental error.

The initial BW was used as a covariate for the statistical analysis of ADG.

The DM and nutrient intake, digestibility, feed efficiency, carcass gain, and methane emission data were analyzed with starch level and oil inclusion as fixed effects and the residual error as a random effect using PROC MIXED of the SAS statistical software (SAS Inst. Inc., Cary, NC). Homogeneity of the data was verified using the UNIVARIATE procedure of SAS. Studentized residuals were plotted against the predicted values using the plot procedure to analyze data for outliers. The LSMEANS statement of the mixed procedure of SAS was used to calculate mean values. When the treatments were significant, the means were compared with Fisher's tests using the PDIFF option in LSMEANS command. The level of significance used to assess differences among means was $\alpha = 0.05$.

3. RESULTS

There were no interactions between starch level and oil supplementation with regard to intake of DM ($P = 0.67$), forage DM ($P = 0.55$), supplement DM ($P = 0.14$), OM ($P = 0.66$), CP ($P = 0.74$), NDF ($P = 0.50$), EE ($P = 0.47$) and GE ($P = 0.68$). There was no effect of starch or oil on intake of DM, forage DM, supplement DM, OM, CP, NDF, and GE ($P > 0.05$). However, the addition of oil increased the intake of EE ($P < 0.01$) independently of starch level used (Table 2).

There were no interactions between starch level and oil supplementation on digestibility of DM (P = 0.18), OM (P = 0.11), NDF (P = 0.42), and EE (P = 0.14). There was an interaction between starch and oil supplementation on GE digestibility (P < 0.01; Table 2).

Table 2. Effect of supplements containing high or low starch with or without oil (Oil or No Oil) on intake and digestibility in Nellore bulls

	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Intake, % of BW								
DM	2.75	2.49	2.48	2.72	0.19	0.93	0.94	0.24
Intake, kg/d								
DM	7.70	7.69	7.45	7.85	0.47	0.92	0.69	0.67
Forage DM	6.28	6.14	5.94	6.39	0.48	0.93	0.76	0.55
Supplement DM	1.41	1.54	1.51	1.45	0.05	0.95	0.54	0.14
OM	7.12	7.06	6.87	7.21	0.43	0.90	0.74	0.66
CP	1.04	1.07	1.03	1.09	0.05	0.89	0.44	0.74
NDF	3.96	3.86	3.83	4.13	0.28	0.80	0.74	0.50
EE	0.28	0.13	0.28	0.12	0.01	0.71	< 0.01	0.47
GE, *Mcal/d*	35.11	34.13	33.93	34.70	2.08	0.88	0.96	0.68
Total digestibility, g/kg DM								
DM	630.80	640.40	594.60	654.80	1.74	0.55	0.08	0.18
OM	668.20	676.60	627.40	691.20	1.54	0.42	0.04	0.11
NDF	601.70	643.10	594.20	678.10	2.50	0.59	0.03	0.42
EE	760.10	575.90	574.40	642.20	7.89	0.47	0.48	0.14
GE	649.00^{ab}	628.20^{bc}	608.00^{c}	664.50^{a}	1.02	0.82	0.11	< 0.01

$^{a–c}$ Means within a row with different superscripts differ (P < 0.05).

[1]High starch: 136 g/kg of starch in DM supplement.

[2]Low starch: 41.5 g/kg of starch in DM supplement.

Thus, animals supplemented with high starch and oil showed greater digestibility of GE than those supplemented with less starch and oil. Independently of starch level utilized, the addition of oil decreased the digestibility of OM (P = 0.04) and NDF (P = 0.03; Table 2). In relation to animal performance, there were no main effects of starch level or oil, or interaction between starch × oil for initial BW (P = 0.10), final BW (P = 0.94), ADG (P = 0.40), FE (P = 0.37), and CrG (P = 0.38; Table 3).

Table 3. Effect of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on initial and final BW, ADG, feed efficiency (FE), and carcass gain (CrG) of young Nellore bulls on pasture in the growing phase

	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Initial BW, *kg*	239.45	259.11	257.55	246.66	8.49	0.74	0.61	0.10
Final BW, *kg*	352.47	359.39	350.01	357.68	4.65	0.65	0.14	0.94
ADG, *kg/day*	0.89	1.03	0.92	0.97	0.04	0.77	0.11	0.40
FE[3]	0.116	0.134	0.124	0.124	0.01	0.90	0.35	0.37
CrG[4]	0.46	0.52	0.47	0.49	0.02	0.83	0.11	0.38

[1]High starch: 136 g/kg of starch in DM supplement.
[2]Low starch: 41.5 g/kg of starch in DM supplement.
[3]Feed efficiency = kg ADG/kg DM intake.
[4]Carcass gain = g/day.

Enteric methane emissions, expressed in $g \cdot d^{-1}$ (P = 0.77), $kg \cdot yr^{-1}$ (P = 0.77), $g \cdot kg^{-1}$ DMI (P = 0.53), and $g \cdot kg^{-1}$ CrG (P = 0.31), were not affected by the addition of oil or by the starch level supplemented to the animals. However, there was an interaction between starch level and oil on methane emissions when enteric methane emission was corrected for NDF intake (P = 0.04). Additionally, oil decreased enteric methane emissions relative to GE (P = 0.04) and EE intake (P < 0.01) for animals fed high- or low-starch supplement (Table 4).

Table 4. Effect of supplements containing high or low starch with or without oil (Oil or No Oil) on enteric methane emission of young Nellore bulls on pasture in the growing phase

CH_4 outputs[3]	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
g/d	117.74	127.63	114.61	120.48	6.95	0.48	0.28	0.77
kg/yr	42.97	46.58	41.83	43.97	2.53	0.48	0.28	0.77
g/kg DMI	15.36	17.14	15.45	15.44	1.37	0.57	0.54	0.53
g/kg NDFI	29.95^{b}	39.18^{a}	30.11^{b}	29.42^{b}	2.14	0.05	0.07	0.04
% of GEI	3.37	4.38	3.39	3.49	0.23	0.10	0.04	0.08
g/g EEI	0.42	1.10	0.40	0.99	0.03	0.16	< 0.01	0.24
g/kg CrG	257.75	246.33	228.51	257.80	18.93	0.65	0.64	0.31

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[1]High starch: 136 g·kg^{-1} of starch in DM supplement.
[2]Low starch: 41.5 g·kg^{-1} of starch in DM supplement.
[3]NDFI = NDF intake; GEI= GE intake; EEI = ether extract intake; DMI = DM intake; CrG = carcass gain.

4. DISCUSSION

This study evaluated the effects of starch-based supplementation level combined with oil on intake, digestibility, performance, and methane emissions of Nellore bulls fed *Brachiaria brizantha* cv. Xaraés. The addition of oil to the diet, independent of starch level utilized, was associated with reduced methane emissions, but only when enteric methane was corrected for GE and EE intake. Animal performance, in turn, was compromised by the addition of oil in the growing phase, independent of starch level used.

Fats can exert adverse effects on intake, digestibility, rumen fermentation, methane emissions, and performance of animals depending upon the concentrations and type of fats in diets (Coppock and Wilks, 1991; Machmüller, 2006). In the current study the effects of supplemental fat on intake of DM, forage DM, supplement DM, OM, CP, NDF, and GE were not significant. This is consistent with previous observations of no reduction in DM intake when supplementing corn oil (2.36%; Duckett et al., 2002), yellow grease (2 to 6%; Zinn et al., 2000), or high-oil corn (Eibs et al., 2000; Duckett et al., 2002) for steer diets. The amount of fat to include in the diet should be determined based on the desirable energy concentration, in association with the other ingredients in the diet (e.g., high- *vs.* low-quality forage; saturation level of the fat and fiber level in the diet) (Hall and Eastridge, 2014).

Animals supplemented with oil in this study showed greater EE intake than those supplemented without oil, 37 and 16 g per kg DM, respectively. Diets containing 70 g of EE per kg DM or more can cause feed degradation, be toxic to ruminal microorganisms, adhere to food particles and create a physical barrier that prevents the action of microorganisms and microbial enzymes especially if there are great proportions of unsaturated fatty acids contained within the EE (Palmquist and Jenkins, 1980; Sullivan et al., 2004). Therefore, it can be concluded that there was no difference in intake due to the amount of oil consumed by animals.

In relation to the digestibility of the DM and nutrients, there was no difference between SH and corn. On the other hand, supplementation with oil decreased the total digestibility of OM (5.3%) and NDF (9.5%) components, for both the animals fed the low and high-starch supplement. This is likely due to a decrease in the number of rumen protozoa, various bacteria including fibrolytic bacterial populations and decreased activity of fiber degrading enzymes (Hristov et al., 2009; Huws et al., 2010; Patra and Yu, 2013; Yang et al., 2009). Fibrolytic bacteria are among the most sensitive to inhibition by dietary fats (Nagaraja et al., 1997).

Different forms of fat supplementation can affect NDF digestibility to a different extent. Oil seeds have less of a negative effect on fiber digestibility than oil supplementation. Oils may be readily adsorbed by fiber components of feeds in greater amounts than fats released from digestion of oil seeds, resulting in greater inhibitory effect on fiber digestion (Patra, 2013). In this study the form of fat used was milled grain soybeans. This form of fat substantially decreased the digestibility of OM and NDF, but did not exert detrimental effects on intake of animals.

In this study there were no differences due to the main effect of starch source or an interaction between starch source and the presence of oil on final BW, ADG, feed efficiency and CrG. This is in line with the notion that in high-forage beef cattle diets, the nutritive value of SH is similar to that of corn (Anderson et al., 1988), and that possible changes in ruminal fermentation associated with the different carbohydrate sources did not affect efficiency of feed use for growth.

Effects of SH on animal performance may be related to inclusion rate. At low inclusion rates, SH do not compromise performance because SH are fairly digestible compared with corn. Thus, when included at low percentages of diet DM in concentrate diets, SH may reduce metabolic upsets, thereby increasing energy availability from other dietary components. In the case of forage-based diets, SH probably do not decrease fiber digestion as do starch-containing

feedstuffs, such as corn. Consequently, energy intake may be enhanced by feeding SH compared with cereal concentrates (Ludden et al., 1995).

In relation to the performance characteristics, supplementation with addition of oil was compromised independent of starch level used. There was a numerical decrease of 9.5% (ADG) and 8.0% (CrG) for animals fed with oil compared to without oil. The inclusion of oil in the diet (40-50 g EE/kg DM) for cattle has the capacity to reduce digestibility and thus may affect animal production performances (Chuntrakort et al., 2014). In this study, the supplementation with oil decreased the digestibility of OM (5.3%) and NDF (9.5%), indicating that feeds containing oil could contribute to the negative effect on digestibility those components. Our data agree with previous findings (Grainger et al., 2010, Lovett et al., 2003 and

McGinn et al., 2004) in that the addition of oil reduces digestibility.

The medium- and long-chain fatty acids can be inhibitory for gram positive rumen bacteria, including ruminococcus cellulolytic bacteria, which could explain the low digestibility of the high roughage diet in the oil supplements (Dohme et al., 2000 and Martin et al., 2008). The mechanism of reduced fiber digestibility caused by oil supplementation may also be related to the hydrogenation process of unsaturated fatty acids in the rumen. If the ability of the microorganisms to saturate the fatty acids were exceeded, then the unsaturated fatty acids would accumulate and interfere with microbial digestion (NRC, 2000).Therefore, diets containing oil ingredients should be utilized after considering the effects of the optimum level of oil seed supplementation on animal performance.

Enteric methane emission expressed in g/d, kg/yr, g/kg of DMI, and g/kg of CrG was not affected by supplementation with oil or by starch level. However, there was an interaction between starch level and oil on methane emissions when enteric methane emission was corrected for NDFI. Supplement with high starch and without oil increased methane production by 23.9% compared to other supplements. The results demonstrated that the methane-suppressing effects of fats might be more marked with high concentrations of starch in diets. This probably occurs because fats may readily be adsorbed on to the fiber particles, which may lower the effective inhibitory concentrations in the rumen fluid or adsorption onto bacteria including methanogens (Patra, 2013). Consequently, this probably decreases the inhibitory effect of fats on methanogens in low-starch supplements. The *in vivo* study by Machmüller et al. (2003) revealed that the extent of inhibition of methanogenesis by fat might be lowered with high content of fibrous carbohydrate in diets.

On the other hand, regardless of starch level, the results this study demonstrated that the inclusion of oil in supplements of cattle on pasture mitigates methane emission, when was corrected for EEI (g CH_4/g of EEI). The oil supplementation decreased enteric methane emission significantly compared with supplements without oil, which was consistent with previous reports (Chuntrakort et al., 2011, Grainger et al., 2010 and Jordan et al., 2006). Possible reasons for methane suppression by fat sources include a reduced supply of fermentable organic matter, depressed digestibility and direct inhibitory effects against methane-producing microbes (Machmüller et al., 2003).

The inhibitory response of fats on methane production depends upon concentration, type, fatty acid composition of fats, and nutrient composition of diets (Beauchemin et al., 2008; Machmüller, 2006). Greater concentrations of fats do substantially decrease methane production, but often exert detrimental effects on digestibility and fermentation of feeds including animal performance (Patra, 2013).

The average energy lost in the form of methane emission (expressed as consumed energy) was 3.4% in the animals receiving the supplements with oil and 3.9% in the supplements without oil. This value is less than the value reported by the Intergovernmental Panel on Climate Change (IPCC, 2006) for animals consuming less than 90% concentrate in the diet (6.5% of GEI). This loss of energy may have indirect but significant financial implications for production system, because it coincides with greater energy-use efficiency of the feed by the animal, and may provide an incentive for adopting mitigation strategies that can reduce methane output and improve animal performance (Mc Geough et al., 2010).

The inhibitory effect of fat on enteric methane emissions has been reported in the majority of studies though the extent of inhibition varies (Brask et al., 2013; Grainger and Beauchemin, 2011). Dietary fat inhibits methanogenesis by reducing the metabolic activity and numbers of ruminal methanogens and protozoa, diminishing the quantity of feeds fermented in the rumen, and through biohydrogenation (an alternate hydrogen sink) of unsaturated fatty acids (Beauchemin et al., 2009; Johnson and Johnson, 1995; Lillis et al., 2011).

Although our study found differences in methane emissions over the fiber and gross energy intake, emission per kg of carcass gain was not affected. In this study, the mean calculated value of enteric methane emission for the treatments employed here was 43.8 kg CH_4/yr. This estimate is below the estimate of 56 kg CH_4/yr made by the Intergovernmental Panel on Climate Change (IPCC, 2006) for cattle.

Diet modification is one way in which the cattle industry can reduce its contribution to greenhouse gas emissions. Thus, an ingredient that reduces methane emissions from cattle fed pasture-based diets could have an important impact on reducing the emissions in tropical regions. This study demonstrates that oil can be added to starch-based supplements to reduce methane emissions as a percentage of GE intake by 12.8%, without impairing animal performance. These reductions in methane are important because total methane per animal and methane relative to GE intake are the approaches used by the IPCC (2006) in calculating methane inventories. Although studies show that diet composition affects the production of GHG by ruminants, the IPCC in 2006, responsible for the development of methodologies for estimating global emission inventories, only makes differentiation between two diets: diets with more than 90% concentrate (3% of GE intake is lost as methane); and diets with less than 90% concentrate (6.5% of GE intake is lost as methane). This cannot be consistent with the conditions observed in the tropical regions of ruminant production systems, which use a low inclusion of concentrate in the diet of animals. Therefore, the interval of 0 to 90% concentrate for estimating the emission of methane by beef cattle is too large. Thus, the results determined in this study, with forage:concentrate ratio (80:20), and average energy lost in the form of methane emissions of 3.65% for animals fed pasture-based diets in tropical regions, may be important for new estimates of the IPCC, for global emission inventories.

5. CONCLUSION

Soybean hulls have an estimated feeding value comparable with corn when supplemented to animals raised on pasture, as indicated by the similar performance of these sources of energy. The use of oil supplementation may be effective to reduce enteric methane emission losses per unit of gross energy and EE intake for growing young Nellore bulls fed *Brachiaria brizantha* cv. Xaraés during the rainy season.

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CHAPTER 4

O artigo a seguir está redigido conforme normas de publicação do *Journal of Animal Science* exceto o posicionamento das tabelas.

METHANE EMISSIONS FROM FINISHING NELLORE BULLS ON PASTURE FED WITH TWO LEVELS OF STARCH-BASED SUPPLEMENT WITH OR WITHOUT OIL

ABSTRACT: Ruminant livestock produce about 80 million tons of methane (CH_4) annually and represents one of the few sources of CH_4 that can be manipulated. This study evaluated the combined effects of high- or low-starch supplements with or without oil on intake, digestibility, performance, and CH_4 emissions of finishing Nellore bulls (n = 44, initial BW = 414 ± 12 kg) fed *Brachiaria brizantha* cv. Xaraés during the dry season. The experimental design was completely randomized in a 2 × 2 factorial arrangement (high or low starch, with or without a source of oil). There were no interactions between starch level and oil supplementation with regard to intake of DM (P = 0.90), forage DM (P = 0.95), supplement DM (P = 0.87), OM (P = 0.88), CP (P = 0.96), NDF (P = 0.65), EE (P = 0.56) and GE (P = 0.82). However, there was main effect of starch and oil on intake of EE (P < 0.01). There were no interactions between starch level and oil supplementation on digestibility of DM (P = 0.12), OM (P = 0.13), NDF (P = 0.12), CP (P = 0.06), EE (P = 0.28) and GE (P = 0.82). Independently of starch level utilized, the addition of oil decreased the digestibility of NDF (P = 0.03), and increased EE digestibility (P < 0.01). In relation to animal performance, there were no interactions between starch level and oil for initial BW (P = 0.63), final BW (P = 0.37), ADG (P = 0.41), FE (P = 0.47), HCW (P = 0.83), dressing (P = 0.41), carcass gain (P = 0.98), fat depth (P = 0.36) and LM area (P = 0.91). However, the addition of oil increased the fat depth (P = 0.01) independently of starch level used. There was no interaction between starch-based supplementation level and oil on CH_4 emissions when expressed in g/d (P = 0.78), kg/yr (P = 0.78), g/kg DMI (P = 0.81), g/kg OMI (P = 0.82), g/kg NDFI (P = 0.61), % of GEI (P = 0.85), g/g EEI (P = 0.23), g/kg ADG (P = 0.48), and g/kg of carcass gain (P = 0.85). Therefore, the addition of oil in supplements, independent of starch level used, was associated with reduced CH_4 emissions expressed in g/d (P = 0.04) and kg/yr (P = 0.04). Additionally, oil decreased enteric CH_4 emissions relative to GE (P = 0.02) and EE (P < 0.01) intake, and ADG (P = 0.02) for animals fed high- or low-starch supplement. Soybean hulls (SH) were similar in energy value to corn when used to supplement the grazing beef animal. The use of oil supplementation may be used to reduce CH_4 losses from beef cattle raised on tropical pasture.

Key words: *brachiaria*, greenhouse gases, lipids, ruminant, soybean hulls

1. INTRODUCTION

Methane emissions (CH_4) from enteric fermentation could increase by 31% between 1990 and 2030 (Valin et al., 2013), and represents an energy loss to the animal ranging from 2 to 12% of GE intake (Johnson and Johnson, 1995). Therefore, the challenge is to develop diets and handling strategies to mitigate CH_4 emissions (e.g., CH_4/kg of meat), increase production efficiency and decrease livestock contribution to global warming (McGeough et al., 2010).

The utilization of nonstructural carbohydrates in ruminant diets may increase amylolytic bacteria and decrease methanogen and fibrolytic bacteria numbers (Martin et al., 2010). Similarly, in contrast to fiber, starch fermentation can favor the production of propionate, creating an alternative hydrogen sink to methanogenesis (Bannink et al., 2008). However, there is substantial variation in the fermentation kinetics of different starch sources, and hence leads to variability in the quantity of CH_4 produced (Popova et al., 2013).

Methane emission could also be reduced by the addition of fats in ruminant diets due to lower the quantity of OM fermented in the rumen and by influencing the microbial activity and rumen ecosystem and, to a very minor extent, by biohydrogenation, once some microorganisms in the rumen use hydrogen to hydrogenate the double bonds of unsaturated fatty acids (Johnson and Johnson, 1995).

Limited information is available on combined effects of different carbohydrate forms and oil source on animal performance and CH_4 emissions from beef cattle on tropical pasture. The hypothesis of the present study is that fat supplementation as a source of energy could reduce CH_4 emissions without affecting animal performance, independently of starch level utilized. This study evaluated the combined effects of high- or low-starch supplements and oil on intake, digestibility, performance, and CH_4 emissions of young Nellore bulls fed *Brachiaria brizantha* cv. Xaraés during the finishing phase.

2. MATERIALS AND METHODS

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal) of the Faculty of Agriculture and Veterinary Sciences – São Paulo State University (UNESP) – Jaboticabal campus (protocol number 021119/11).

Animals and management

The experiment was conducted at the UNESP (Jaboticabal, SP, Brazil) from May to October 2013, in the dry season. Under the international Köppen classification this climate is characterized as tropical type AW with summer rains and relatively dry winter; the local altitude is 595 m, at 21°15'22" south latitude, 48º18'58" west longitude.

Forty-four Nellore bulls were used in the experiment, with an average age of 20 mo and initial body weight (IBW) = 414 ± 12 kg. Initially, the animals were weighed, identified, and treated against ecto- and endoparasites by administration of ivermectin 1% (Ivomec®, Merial, Paulínea, BR), and allocated into 12 paddocks of 1.8 ha, consisting of *Brachiaria brizantha* cv. Xaraés. The animals were distributed in a completely randomized design (3 animals per paddock and 3 paddocks per treatment). The experimental period lasted 140 d, divided into an adaptation period of 28 d and four periods of 28 d each.

Grazing method used was continuous stocking with variable stocking rate. Forage height was randomly measured every 28 d by eighty points using a graduated stick, in each paddock (Barthram, 1985). Samples to address herbage chemical composition were obtained by hand plucking (Johnson, 1978). Hand plucking was performed on the same days as the estimation of DMI, described later. The simulation of grazing per paddock was performed every 28 d.

The diets used consisted in *Brachiaria brizantha* cv. Xaraés pasture supplemented with two levels of starch, with or without a source of oil. The supplements were corn associated or not with ground soybean, and SH associated or not with ground soybean. Crude glycerin was used in all supplements to replace (28% of DM) corn or SH. This is a byproduct from the biodiesel agroindustry and can be used in ruminant diets without compromising intake and performance (Drouillard, 2012; Parsons et al., 2009). Crude glycerin (83.90% glycerol, 1.75% ether extract, 4.30% ash, and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil). The proportion of ingredients and chemical composition of supplements are presented in Table 1.

Animals were supplemented at the rate of 1000 g/100 kg of BW, daily, at 1000 h, with 3 m of feed bunk line, and had *ad libitum* access to water and shade. The amount of supplement provided was calculated to meet the requirements for average daily gain of 1.0 kg/d, according Valadares Filho et al., 2010. Every 28 d the animals were weighed, after a 16-h withdrawal period from feed and water, and this BW was used to adjust the amount of supplement.

Average daily gain (ADG) was obtained by weighing the animal at the beginning and the end of the experiment, always after a 16-h withdrawal period from feed and water.

Table 1. The ingredient proportions and chemical composition of supplements and pasture (% DM basis)

Item	High Starch		Low Starch		Pasture[1]
	Oil	No Oil	Oil	No Oil	
Ingredient proportions					
Ground corn[*]	18.5	31.0	0.00	0.00	-
Soybean meal	0.00	38.5	0.00	37.0	-
Soybean hulls	0.00	0.00	18.5	32.5	-
Ground soybean[*]	51.0	0.00	51.0	0.00	-
Crude glycerin	28.0	28.0	28.0	28.0	-
Commercial premix[2]	2.50	2.50	2.50	2.50	-
Chemical composition					
Dry matter	90.2	89.3	90.3	89.4	-
Organic matter	92.3	92.2	91.7	91.3	92.6
Crude protein	22.9	22.3	23.9	23.6	9.01
NDF	12.7	11.1	21.9	27.1	65.8
Starch[3]	17.20	24.69	4.45	3.29	-
Ether extract	12.4	3.62	11.8	2.58	2.12
Gross energy, *Mcal/kg DM*	5.08	4.62	4.98	4.45	4.02

[1]Average of samples obtained by technique of simulated grazing in 5 periods.

[2]120 g calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1.220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, 300 mg fluorine; no additive.

[3]Calculated based on ingredient values from Valadares Filho et al., 2010;

[*]Ground in a hammermill fitted with screen size of 3.0 mm (fine).

Carcass gain (CrG) was determined via the comparative slaughter technique. Eight animals were slaughtered at a commercial beef plant and served as the reference group at the beginning of the experiment as the initial dressing percentage (DP; 52.02%), which estimated the initial carcass weight to obtain the CrG at the end of experiment. The 8 animals slaughtered were taken from a random sample, with 2 animals per treatment.

After 140 d of feeding, all the animals were slaughtered at commercial beef plant with 533 ± 37 kg of shrunk body weight (SBW). Preharvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997).

After the slaughter, the carcass was weight and all carcasses were refrigerated at 4 °C for approximately 24-h. After the postmortem chill period, 12th fat depth and 12th rib *longissimus* muscle area (LM area) were measured on the left side of each carcass. *Longissimus* muscle areas were traced on transparencies and measured later with a planimeter and fat depth measurements were taken 3/4 the length ventrally over the *longissimus* muscle (Greiner et al., 2003). Carcass gain was obtained using the final hot carcass weight (HCW) minus initial estimated carcass weight (initial BW × DP of initial reference group) per number of days feeding. Dressing percent was calculated using HCW divided by final SBW and then multiplying the result by 100.

Proximate analysis

For proximate analysis, the sample of ingredients of supplements, forage, and feces were dried at 55ºC for 72-h. Samples were then ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen, and analyzed for DM (method 934.01), OM (method 942.05), and EE (method 920.85) according to the Association of Official Analytical Chemists (AOAC, 1995). Concentrations of N in each sample were determined by rapid combustion (850°C), conversion of all N-combustion products to N_2, and subsequent measurement by thermoconductivity cell (Leco model FP-528; LECO Corporation, St. Joseph, MI). Crude protein was calculated as the percentage of N in the sample multiplied by 6.25. The GE content of supplements, forage, and feces was determined using an adiabatic bomb calorimeter (model 6300; Parr Instrument Company, Moline, IL). Analyses for NDF were conducted following Van Soest et al. (1991) and adapted for the Ankom^{200} Fiber Analyzer (Ankom Technology, Fairport, NY). Heat-stable α-amylase was included in the NDF solution, without added sodium sulfite.

Intake estimation

Thirty-six animals were used to estimate intake and nutrient digestibility. Lignin isolated, purified, and enriched from *Eucalyptus grandis* (LIPE) and indigestible NDF (iNDF)

were used to estimate the excretion of fecal matter (as dry weight) and forage intake, respectively.

Lignin isolated, purified, and enriched from *Eucalyptus grandis* was provided for 7 d by oral administration of a 500 mg bolus, with 4 d to stabilize fecal excretion of the marker, and in the last 3 d for sample collection (Saliba, 2005). Fecal samples were collected during 3 d, directly from the rectum, at 1600, 1100 and 0700 h on the first, second and third day of collection, respectively. The fecal samples were dried at 55°C for 72-h and ground in a Wiley mill (Thomas Scientific) to pass through a 1-mm screen and composited proportionately on each of 3 d of sampling, within each animal, based on fecal dry weights. Approximately 10 g of each composited sample of feces was sent to the Federal University of Minas Gerais (Belo Horizonte, MG, Brazil) to estimate the total daily fecal output by 2 methods of LIPE measurement as described by Saliba (2005). Individual concentrate intake was estimated by dividing the total concentrate provided by the number of animals in each paddock.

The individual intakes of forage were estimated using the internal marker iNDF. The samples of feces, forage, and concentrate were placed in Ankom bags (Filter bag F57; Ankom Technology Corporation) and incubated in the rumen of a fistulated Nellore animal for a period of 288-h (Valente et al., 2011). When the bags were withdrawn from the rumen, they were soaked in water for 30 min and gently washed by hand under running water until the wash water ran clear. The bags were then placed in an Ankom^{200} fiber Analyzer (Ankom Technology Corporation), as described by Van Soest et al. (1991), and the iNDF was determined by weighing the bags with a digital scale after drying them in an oven, first at 55°C for 72-h and then at 105°C for 12-h. The residue was considered the iNDF. Individual forage intakes were estimated by subtracting marker excretion from the concentrate from the total iNDF excretion and dividing that difference by the concentration of the marker in the forage.

Methane Measurements

Methane emissions were assessed using the sulfur hexafluoride (SF_6) tracer technique (Johnson et al., 1994), in which each animal was sampled daily for 6 consecutive 24-h days, beginning on d 65 of feeding. Thirty-six animals were fitted with gas collection halters at 14 d before CH_4 sampling to allow animals to adapt and facilitate sampling.

The release rate (RR) of the gas from a permeation tube is known before its insertion into the rumen. The permeation tubes were maintained in a water bath at 39°C and weighed in

the laboratory for 7 wk. The average RR was similar among the treatments (RR 1.53 ± 0.3 mg SF_6/d, mean ± SD). Brass permeation tubes filled with SF_6 and known RR were administered orally to each of the 36 animals 72-h before CH_4 sampling to allow the tracer gas to equilibrate in the rumen. The animals were fitted with gas collection halters connected to pre-evacuated polyvinyl chloride (PVC) canisters designed to fill halfway over 24-h. Eructated gas sampling started at 0700 h daily, when the animals were removed from the paddocks, and was conducted at the management center (stockyard) to facilitate sampling.

The collection canisters were located above each animal to reduce the risk of equipment damage and were connected to the halter by tubing inside airline flexible-coil tubing. Collection canisters, constructed of PVC pipe, were attached to a vacuum pump in the laboratory to create a negative pressure. As the vacuum in the sampling canister was slowly dissipated, the negative pressure steadily drew the sample of air from around the mouth and nose of the animal. The pressure of the canister, removed from the animal, was measured after 24-h of collection and if the final pressure was out of the expected range, the halter was preventively replaced. If the final pressure was above the expected range, the halter was probably blocked or disconnected; if the final pressure was below the expected range, possibly there was a leak in the system. In both situations a new halter was placed on the animal with an average absorption rate within the stipulated range (fill halfway over 24-h).

After sampling (approximately 30 min), each animal was brought to the original paddock for feeding. The pressure readings were recorded, and the canisters were pressurized using pure N_2. Additional canisters were placed near the experimental pasture to monitor background levels of CH_4 and SF_6 daily during each sampling period. These values were subtracted from the animals' values to calculate the net output in the expired breath. The concentrations of CH_4 and SF_6 in the collection tubes were measured at the Laboratory of Animal Nutrition – UNESP (Jaboticabal, SP, Brazil) using an gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) equipped with column Porapak Q (2m × 3mm i.d., 80 to 100 mesh, Shimadzu, Kyoto, Japan), flame ionization detector for CH_4, and electron capture detector for hexafluoride, as described by Johnson et al. (1994).

Methane flux produced by animals was calculated in relation to the SF_6 tracer gas flux from a permeation capsule lodged in the rumen minus the basal CH_4 concentration in the air (Westberg et al., 1998).

The following equation was used:

$Q_{CH4} = Q_{SF6} \times ([CH_4]_y - [CH_4]_b)/[SF_6]$,

in which Q_{CH4} = CH_4 emission tax by animal, Q_{SF6} = know SF_6 emission tax from capsule in rumen, $[CH_4]_y$ = CH_4 concentrations in collection apparatus, $[CH_4]_b$ = basal CH_4 concentration, and $[SF_6]$ = SF_6 concentration in collection apparatus.

Statistical Analysis

The experimental design was completely randomized in a 2 × 2 factorial arrangement (high or low starch, with or without a source of oil). Each paddock was considered as the individual experimental unit (3 animals per paddock and 3 paddocks per treatment).

The mathematical model was represented by:

$Y_{ijk} = \mu + S_i + O_k + (S_i \times O_k) + e_{ijk}$,

in which Y_{ijk} = observation of paddock j subject to starch i at oil inclusion k, μ = the overall mean, S_i = effect of starch i = 1 and 2, O_k = effect of oil inclusion k = 1 and 2, $S_i \times O_k$ = interaction between starch i and oil inclusion k, and e_{ijk} = the residual experimental error. The interaction between the covariate and variables was tested and removed from the statistical model if not significant at $P < 0.05$. The initial BW was used as a covariate for the statistical analysis of final BW, HCW, and fat depth.

The DM and nutrient intake, digestibility, performance and CH_4 emission data were analyzed with starch level and oil inclusion as fixed effects and the residual error as a random effect using PROC MIXED of the SAS statistical software (SAS Inst. Inc., Cary, NC). Homogeneity of the data was verified using the UNIVARIATE procedure of SAS. Studentized residuals were plotted against the predicted values using the plot procedure to analyze data for outliers. The LSMEANS statement of the mixed procedure of SAS was used to calculate mean values. When the treatments were significant, the means were compared with Fisher's tests using the PDIFF option in LSMEANS command. The level of significance used to assess differences among means was $\alpha = 0.05$.

3. RESULTS

There were no interactions between starch level and oil supplementation with regard to intake of DM (% of BW, $P = 0.98$; Total, $P = 0.90$), forage DM ($P = 0.95$), supplement DM ($P = 0.87$), OM ($P = 0.88$), CP ($P = 0.96$), NDF ($P = 0.65$), EE ($P = 0.56$) and GE ($P = 0.82$).

There was no effect of starch or oil on intake of DM, forage DM, supplement DM, OM, CP, NDF, and GE ($P > 0.05$; Table 2).

Table 2. Effect of supplements containing high or low starch with or without oil (Oil or No Oil) on intake and digestibility in Nellore bulls

Item	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Intake, % of BW								
DM	2.56	2.52	2.61	2.55	0.10	0.69	0.66	0.98
Intake, kg/d								
DM	12.70	12.81	12.63	12.65	0.37	0.75	0.86	0.90
Forage DM	7.73	7.70	7.77	7.70	0.39	0.96	0.90	0.95
Supplement DM	4.97	5.11	4.86	4.94	0.14	0.37	0.48	0.87
OM	11.76	11.85	11.67	11.66	0.34	0.68	0.90	0.88
CP	1.84	1.83	1.87	1.86	0.04	0.50	0.90	0.96
NDF	5.70	5.74	6.07	6.34	0.25	0.09	0.55	0.65
EE	0.78	0.34	0.74	0.29	0.01	< 0.01	< 0.01	0.56
GE, *Mcal/d*	56.43	54.65	55.52	53.04	1.51	0.42	0.19	0.82
Total digestibility, g/kg DM								
DM	652.40	642.42	646.21	676.20	1.15	0.26	0.41	0.12
OM	701.74	683.43	690.12	725.40	1.62	0.37	0.61	0.13
NDF	541.76	553.59	533.94	602.71	1.63	0.24	0.03	0.12
CP	610.24	555.43	622.59	640.85	1.74	0.02	0.32	0.06
EE	697.25	540.52	665.14	579.03	3.10	0.92	< 0.01	0.28
GE	637.57	613.59	643.24	626.29	1.56	0.57	0.22	0.82

[1]High starch: 209.4 g/kg of starch in DM supplement.
[2]Low starch: 38.7 g/kg of starch in DM supplement.

However, there was effect of starch and oil on intake of EE ($P < 0.01$). The addition of oil increased the intake of EE ($P < 0.01$) independently of starch level used, and animals supplemented with high starch showed greater intake of EE ($P < 0.01$) than those supplemented with less starch (Table 2). There were no interactions between starch level and oil supplementation on digestibility of DM ($P = 0.12$), OM ($P = 0.13$), NDF ($P = 0.12$), CP ($P = 0.06$), EE ($P = 0.28$) and GE ($P = 0.82$). There was effect of high starch supplement on digestibility of CP ($P = 0.02$). Independently of starch level utilized, the addition of oil decreased the digestibility of NDF ($P = 0.03$), and increased of EE ($P < 0.01$; Table 2).

Table 3. Effect of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on initial and final BW, ADG, feed efficiency (FE), HCW, dressing, carcass gain (CrG), fat depth, and LM area of Nellore bulls in pasture

Item	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Initial BW, *kg*	409.33	428.34	405.00	412.00	12.03	0.41	0.31	0.63
Final BW, *kg*	540.03	528.77	532.06	530.47	5.39	0.56	0.26	0.37
ADG, *kg/day*	0.89	0.82	0.83	0.82	0.03	0.41	0.32	0.41
FE[3]	0.070	0.065	0.066	0.065	0.003	0.59	0.36	0.47
HCW, *kg*	307.72	306.32	307.09	307.11	3.50	0.98	0.84	0.83
Dressing, *%*	57.07	57.73	57.87	57.93	0.34	0.18	0.33	0.41
CrG[4]	0.671	0.677	0.627	0.632	0.02	0.08	0.80	0.98
Fat depth, *mm*	4.32	2.91	4.08	3.32	0.35	0.81	0.01	0.36
LM area, cm^2	81.47	85.93	80.33	85.89	4.85	0.90	0.33	0.91

[1]High starch: 209.4 g/kg of starch in DM supplement.
[2]Low starch: 38.7 g/kg of starch in DM supplement.
[3]Feed efficiency = kg ADG/kg DM intake.
[4]Carcass gain = g/day.

In relation to animal performance, there were no interactions between starch level and oil for initial BW, final BW, ADG, FE, HCW, dressing, CrG, fat depth and LM area (P = 0.36). However, the addition of oil increased the fat depth (P = 0.01) independently of starch level used (Table 3).

Enteric CH_4 emissions from Nellore bulls expressed in g/d, kg/yr, g/kg DMI, g/kg OMI, g/kg NDFI, % of GEI, g/g EEI, g/kg ADG, and g/kg CrG were not affected (P = 0.23) by interaction between oil and starch level supplements (Table 4).

Table 4. Effect of supplements containing high or low starch with or without oil (Oil or No Oil) on enteric methane emission of Nellore bulls in pasture

CH_4 outputs [3]	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
g/d	114.61	145.15	115.92	139.79	11.78	0.86	0.04	0.78
Kg/yr	41.83	52.98	42.31	51.02	4.29	0.86	0.04	0.78
g/kg DMI	9.07	11.32	9.25	11.05	0.91	0.96	0.05	0.81
g/kg OMI	9.81	12.24	10.02	11.99	0.99	0.98	0.05	0.82
g/kg NDFI	20.44	25.46	19.43	22.10	2.28	0.36	0.13	0.61
% of GEI	2.04	2.65	2.10	2.63	0.20	0.91	0.02	0.85
g/g EEI	0.14	0.41	0.16	0.48	0.02	0.09	< 0.01	0.23
g/kg ADG	130.16	178.86	142.70	170.25	14.32	0.89	0.02	0.48
g/kg CrG	172.29	214.07	188.86	223.07	19.73	0.53	0.09	0.85

[1]High starch: 209.4 g/kg of starch in DM supplement.
[2]Low starch: 38.7 g/kg of starch in DM supplement.
[3]NDFI = NDF intake; GEI= GE intake; EEI = ether extract intake; DMI = DM intake; CrG = carcass gain.

However, the addition of oil in supplements, independent of starch level used, was associated with reduced CH_4 emissions expressed in g/d (P = 0.04) and kg/yr (P = 0.04). Additionally, oil decreased enteric CH_4 emissions relative to GE (P = 0.02) and EE (P < 0.01) intake, and ADG (P = 0.02) for animals fed high- or low-starch supplement (Table 4).

4. DISCUSSION

This study evaluated the effects of oil supplementation combined with high- or low-starch on intake, digestibility, performance, and CH_4 emissions of Nellore bulls fed *Brachiaria brizantha* cv. Xaraés during the finishing phase. The addition of oil to the diet, independent of starch level utilized, was associated with increased intake of EE, decreased the digestibility of NDF, and reduced CH_4 emissions. Animal performance, in turn, was not compromised by the addition of oil independent of starch level used.

Animals fed with low-starch (SH) supplement consumed the same amount of DM as those fed high-starch (corn). A greater DMI was expected in difference in NDF content between supplements (24.5 *vs.* 11.9% for low- and high-starch, respectively), but the NDF intake was not different. Although SH has high NDF content, and the experimental supplements that SH was energy source increased the NDF concentration, the digestibility was not affected in these diets. Soybean hulls has a small feed particle size and high specific gravity (Mertens, 1997) resulting in a more rapid ruminal scape and in a reduction of the ruminal fill (Iraira et al., 2013; Nakamura and Owen, 1989).These results are consistent with previous studies (Spörndly, 1991; Valk et al., 1990), which showed no differences in DMI between fiber-based and starch based supplements.

Ruminants are able to utilize dietary energy ruminally or post-ruminally. When concentrate supplements are included in pasture diets, associative effects may occur if digestive and metabolic interactions between them change the intake of energy (Dixon and Stockdale, 1999; Galloway et al., 1993). Starch-based supplementation has been shown to improve performance; however, forage intake can be negatively affected (Caton and Dhuyvetter, 1997; Olson et al., 1999). This has been attributed to a reduction in ruminal pH, which may decrease the activity or number of cellulolytic bacteria, reduce the rate of fiber digestion of pasture, and therefore may explain differences in pasture DMI of ruminants on pasture (Delahoy et al, 2003; Dixon and Stockdale, 1999).

On the other hand, supplementation with feedstuffs high in degradable fiber such as SH, may have no negative effects on forage intake, fiber digestion, and may improve performance of cattle compared to of cereal grain supplementation (Bowman and Sanson, 1996; Grigsby et al., 1992; Kunkle et al., 2000). Orr et al. (2008) evaluated ruminal characteristics between steers consuming SH and corn, with no difference detected in ruminal VFA production and ruminal pH when corn was replaced by SH.

Cellulolytic bacteria adhere to fiber and develop colonies before structural polysaccharide degradation can occur (McAllister et al., 1994). Colonization increased with addition of starch but fiber digestion decreased, possibly due to an increase lag time (Firkins et al., 1991). This may explain why greater levels of corn supplementation have a negative effect on forage digestion.

In the current study, supplementation with oil decreased the digestibility of NDF (6.9%), and increased of EE (17.8%), independently of starch level utilized. Fat content of the supplements with oil or no oil was 12.1 and 3.1%, respectively. Although the supplements differed in fat content, the contribution of supplemental fat to total dietary DM intake (Table 2) was only 6.0 and 2.4% for the oil and no oil treatments, respectively. This difference likely would not affect forage DM intake considering the negligible fat contribution from grasses (Harfoot and Hazlewood, 1997), and the 6 to 7% of total dietary intake that needs to be represented by fat in order to negatively affect forage intake in ruminants (Palmquist, 1994; Hess et al., 2008).

Oil supplementation to grazing animals increased diet GE concentration, but the negative effect of oil supplementation on DM intake and digestibility resulted in a reduction of digestible energy (DE) intake (Pavan et al., 2007). Similar results were observed by Scholljegerdes et al. (2004) and McGinn et al. (2004) who found that the decrease in NDF digestibility offset the additional DE supplied by the lipid supplement.

Effects of oil supplementation on fiber digestion depend on the oil source and fatty acid composition, quantity of lipid supplemented, and proportion of forage in the diet (Palmquist, 1984; Jenkins, 1993). Our data agree with previous findings that lipid supplementation compared with animals non-supplemented reduces NDF digestibility of Bermudagrass hay (Grainger et al., 2010; Hall et al., 1990). In contrast, Brokaw et al. (2001) evaluated the effect of lipid supplementation on *in vivo* digestibility of grazing beef cattle; however, the level of soybean oil supplemented was relatively low (0.35 g/kg of BW) and no

effect was observed. In our study, oil supplementation decreased NDF digestibility by 6.9%, for 0.65 g/kg of BW to 1.61 g/kg of BW.

It has previously been demonstrated that supplementing fat increased diet digestibility *in vitro* during sampling times less than 24-h but the response diminished and was reversed by 48-h (Whitney et al., 2000; Brokaw et al., 2002). Similarly effect, Brokaw et al. (2000) noted that heifers supplemented with high-oil corn decreased total tract NDF digestibility than heifers fed conventional corn. In conclusion, digestible energy for diets with supplemental fat would be comparable or greater than the corn-based supplements if one accounts for greater energy value of fatty acids disappearing from the small intestine (Scholljegerdes et al., 2004).

There were no differences due to the main effect of starch source or an interaction between starch source and the presence of oil on final BW, ADG, FE, HCW, dressing, CrG and LM area. These results may be explained by the qualitative characteristics of fiber and by low inclusion rates of SH, which provide an increase in fiber digestion and rumen passage rate (Ipharraguerre and Clark, 2003; Ludden et al., 1995). Digestion is the result of an interaction between digestion rates and permanence at digestion sites (Van Soest, 1994).

On the other hand, animals supplemented with oil in this study, independently of starch level used, showed greater fat depth than those supplemented without oil: 4.2 and 3.1 mm, respectively. This is consistent with previous observations in that lipid supplementation increased external fat deposition in 1.4 mm (Patil et al., 1993).

The addition of fat in beef cattle diet greater than 2 to 3% of DM may decrease digestibility of fibrous feedstuffs such as SH, by inhibiting fibrolytic bacteria (Palmquist, 1988). However, energy provided by fat may compensate for the potential reduction in energy derived from fermentation of SH fiber. Consequently, the intake of DE may be unaffected, or even improved, with greater amounts of dietary fat (Zervas et al., 1998).

Ester linkages of dietary fatty acyl glycerol undergo rapid and extensive hydrolysis by microbial lipolytic enzymes in the rumen to form glycerol and free fatty acids (Jenkins, 1993). Glycerol may then be metabolized by the ruminal microorganisms to produce VFA (Nagaraja et al., 1997). The increased energy density and supply of fatty acids to animals supplemented with oil in our study provided an increase of subcutaneous fat deposition.

In relation to enteric CH_4 emissions, adding ground soybean, a source of unsaturated fat, in supplements for grazing animals was an effective suppressant of CH_4 when expressed in grams per day, kilograms per year, and when corrected as a percentage of GE, and EE

intake, independent of starch level used. Daily CH_4 emissions decreased by 19.09% and CH_4 emissions as a percentage of GE intake decreased by 21.59%.

In a similar study using Nellore bulls fed *Brachiaria brizantha* cv. Xaraés during the rainy season, Jose Neto et al. (2015) demonstrated that oil can be added to starch-based supplements to reduce methane emissions as a percentage of GE intake by 12.8% without impairing animal performance. These reductions in CH_4 are important because total CH_4 per animal and relative to GE intake are the approaches used by the Intergovernmental Panel on Climate Change, in 2006, responsible for the development of methodologies for estimating global CH_4 emission inventories.

Enteric CH_4 is produced under anaerobic conditions in the rumen by methanogenic Archaea, using CO_2 and H_2 to form CH_4, and thus reducing the metabolic H_2 produced during microbial metabolism (McAllister and Newbold, 2008). If H_2 accumulates, the re-oxidation of NADH is inhibited, inhibiting microbial growth, forage digestion, and the associated production of acetate, propionate, and butyrate (Joblin, 1999).

Relationship between DE and ME as well as that of ME and NE can vary considerably among diets with different composition (fiber, starch, fat). One of the forms of energy lost from DE to ME is as CH_4 produced during ruminal fermentation (NRC, 2000). Methane emissions represent a loss of about 5 to 7% of dietary GE (to as low as 3% in cattle fed high-grain diets) and are about 16 to 26 g/kg of dietary DM intake (Hristov et al., 2013).

Feeding grain based diets lowers enteric CH_4 emissions (g/kg DM intake) compared with feeding forage based diets (Johnson and Johnson, 1995). Starch fermentation promotes propionate production in the rumen creating an alternative hydrogen sink to methanogenesis (Huntington et al., 2006), lowers ruminal pH and inhibits growth of rumen methanogens (Van Kessel and Russell, 1996), and decreases rumen protozoal numbers limiting transfer of hydrogen from protozoa to methanogens (Williams et al., 2009).

Soybean hulls contains high amount of cell wall components such as cellulose, rapidly fermentable (Lee et al., 2003). Moe and Tyrell (1979) investigated CH_4 emissions relating to the type of carbohydrates in beef cattle and reported that CH_4 emissions from cellulose was 3 times greater than soluble residue showing that CH_4 emissions were different depending on the type of carbohydrates and among them, cell wall component was most affecting.

In this study, however, there were no differences due to the main effect of starch source or an interaction between starch source and the presence of oil on enteric CH_4

emissions. This is in line with the notion that in high-forage beef cattle diets, the nutritive value of SH is similar to that of corn (Anderson et al., 1988) and that possible changes in ruminal fermentation associated with the different carbohydrate sources did not affect CH_4 emissions of animals.

Nutritional interventions with unprotected lipids reduces CH_4 emissions through multiple mechanisms: by reducing fiber digestion (mainly long-chain fatty acids); reduction of fermentable organic matter (lipids are not a source of energy for rumen bacteria); lowering DMI (if total dietary fat exceeds 6 to 7%); reduction of methanogenic activity due to the presence of medium-chain fatty acids; toxic effects on cellulolytic bacteria and protozoa ; and to a limited extent, through biohydrogenation of PUFAs (Beauchemin et al., 2008; Johnson and Johnson, 1995; McGinn et al., 2004; Nagajara et al., 1997).

Free fatty acids, particularly long chain, unsaturated fatty acids, have been shown to inhibit methanogenesis and increase the production of propionate both *in vitro* and *in vivo* (Czerkawski et al., 1966; Van Nevel and Demeyer, 1981). The fatty-acid-induced decline in CH_4 production was originally attributed to partitioning of available hydrogen between hydrogenation of unsaturated fatly acids and reduction of carbon dioxide (Lennarz, 1966). Long-chain fatty acids are directly toxic to methanogens, protozoa and gram-positive cellulolytic bacteria (Desbois and Smith, 2010; Nagaraja et al., 1997; Zeitz et al., 2013), which accounts for the depressed fiber digestion and reduced ruminal acetate and butyrate production associated with diets containing high concentrations of fatty acids (Van Nevel, 1991). Gram-negative, propionate-producing bacteria, however, are not significantly inhibited by fatty acids (Van Nevel and Demeyer, 1988), thus the reduction in CH_4 emissions observed when fatty acids are included in diets of ruminants results primarily from a shift toward the production of propionate.

The greater inhibitory effect of unsaturated *vs*. saturated fatty acid on rumen microbial activity reported by Palmquist and Jenkins (1980) and Nagaraja et al. (1997) does not appear to apply to CH_4 emission in most studies (Beauchemin et al., 2007; Sauvant et al., 2011; Van Zijderveld et al., 2011) although a greater mitigating effect of polyunsaturated fatty acid was observed in the analysis by Doreau et al. (2011). Biohydrogenation of unsaturated fatty acid can also serve as a hydrogen sink, but it has been suggested that only 1 to 2% of the metabolic hydrogen in the rumen is used for this purpose (Czerkawski and Clapperton, 1984; Jenkins et al., 2008).

In reviewing studies of fat effects on enteric CH_4 emissions of ruminants, Beauchemin et al. (2008) concluded that for every 1% (DMI basis) increase in fat in the diet, CH_4 (g/kg DMI) was reduced by 5.6%. In a more detailed review, compared a total of 67 *in vivo* diets with beef, sheep and dairy cattle, reporting an average of 3.8% (g/kg DMI) less enteric CH_4 with each 1% addition of fat (Martin et al., 2010). In this sense, our data agree with these authors, in that for every 1% (DMI basis) increase in fat in the diet, there was a reduced by 5.0% of enteric CH_4 emissions (g/kg DMI). Assuming that most forage has some fat content and that DMI may be suppressed at fat intakes above 6 to 7%, CH_4 emissions abatements of 10 to 25% are possible with the addition of dietary oils to the diets of ruminants (Beauchemin et al., 2008).

The situations with high emissions per unit of product represent those conditions under which emissions reductions can most likely be achieved without sacrificing overall production of food. Ruminant animals with low levels of production efficiency have relatively high CH_4 emissions per unit of product, because these animals use a large fraction of their feed intake solely for maintenance (FAO, 2006).

Methane emissions increases almost linearly with feed intake and the fraction of ingested energy lost as CH_4 is reduced with higher feed intake. This effect is partially a consequence of an increased rate of rumen passage, and partially a consequence of the type of VFA produced (Monteny et al., 2006). However, CH_4 emission/unit animal product will be reduced if an improvement in animal productivity is achieved (Benchaar et al., 2001).

Additionally, improved grazing management associated with an improvement in the quality of pastures, greater estimated ME content, will also increase animal productivity and decrease CH_4 production (AFRC, 1992; Hegarty, 1999; Shibata and Terada, 2010). Introduction of management intensive grazing increased overall beef production efficiency and that as a result, the CH_4 emissions per unit of product as well as total CH_4 emissions into the atmosphere were reduced (DeRamus et al., 2003).

In this sense, results from our experiment demonstrated that supplementation with oil reduces in 21.8% the enteric CH_4 emission when corrected for ADG (g CH_4/kg of ADG). Increasing calving rate of cows from 55 to 68%, reducing slaughter age from 36 to 28 mo, and reducing mortality from 7 to 4.5% in animals younger than one year of age could reduce CH_4 emissions by 18% in relation to the equivalent level of carcass production in Brazil by 2025 (Barioni et al., 2007).

Livestock farming in Latin America has been criticized because of its large greenhouse gas (GHG) emissions resulting from the use of degraded forage and low efficiency production performance. However, Latin America has a prominent position as an animal protein provider for the world (FAO, 2009). Improvement of food practices to reduce CH_4 emissions per kg of food intake or per kg of product, and the economic viability of their practical applications is an important strategy to consolidate tropical countries as food producers for the world, attending the demands related to land, water, biodiversity conservation, and GHG emissions. Enhancing the efficiency of dietary nutrient use through improved grazing management associated with an improvement in the quality of pastures are the most efficient way of decreasing CH_4 emissions per unit of animal product. Other effective CH_4 mitigation practice include lipid supplementation of the diet, how demonstrated in this study, that ground soybean can be added to high-forage diets to reduce CH_4 emissions from beef cattle on tropical pasture.

5. CONCLUSION

As evaluated in this study, SH was at least equal to corn in energy value as a supplement for grazing cattle. It has the advantages over corn of containing high amounts of digestible fiber rather than starch and, therefore, supplements energy while minimizing changes in ruminal fermentation. A 6.0% supply of lipids from ground soybean significantly decreases by 19.09% the quantity of CH_4 emitted daily without affecting animal performance of Nellore bulls fed *Brachiaria brizantha* cv. Xaraés in the dry season, during the finishing phase.

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CHAPTER 5

O artigo a seguir está redigido conforme normas de publicação do *Journal of Animal Science* exceto o posicionamento das tabelas.

EFFECT OF STARCH-BASED SUPPLEMENTATION LEVEL COMBINED WITH OIL ON FERMENTATION PARAMETERS AND RUMINAL MICROBIOTA OF GROWING NELLORE STEERS ON PASTURE

ABSTRACT: Supplemental with inclusion of high lipid has been used to highly productive animals to satisfy their high-energy requirements. However, the degree of saturation, amount of supplement, and the combination between lipids, starch, and composition of basal diet may cause adverse effects on digestibility of nutrients and rumen fermentation parameters, changing the rumen microbial population. The objective of this study was evaluate the effect of oil supplementation combined with high- or low-starch on intake, nutrient digestibility, rumen fermentation parameters, and rumen microbial profile of young Nellore steers grazing *Brachiaria brizantha cv.* Xaraés during the growing phase. Eight ruminal cannulated Nellore steers (424.8 kg ± 35.5) at 20 mo of age were used in a replicate 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments (high or low starch, with or without a source of oil) and an experimental period of 21 d. The diets used consisted of two levels of starch-based supplement (corn or soybean hulls – SH) with or without a source of oil (ground soybean; GS). The supplements were corn combined with GS; corn without GG; SH combined with GS; and SH without GS. Animals were supplemented at the rate of 500 g/100kg BW. There were no interactions between starch level and oil supplementation on DM and nutrients intake ($P > 0.01$). The addition of oil decreased the intake of DM ($P = 0.01$), forage DM ($P < 0.01$), OM ($P = 0.01$), CP ($P = 0.02$), NDF ($P < 0.01$), and GE ($P = 0.01$), independently of starch level used. Animals fed with low-starch and without oil had greater digestibility of DM ($P < 0.01$), OM ($P < 0.01$), CP ($P < 0.01$), NDF ($P = 0.01$), and GE ($P = 0.01$) than animals fed with other supplements. In relation to rumen fermentation parameters, there were no interactions between starch × oil for pH, NH_3-N, and VFA´s ($P = 0.30$). However, independently of starch level utilized, the addition of oil in the supplements decreased the pH ($P = 0.02$) and NH_3-N ($P = 0.02$). There was interaction between starch and oil supplementation on Entodinium population ($P = 0.04$), with increase of population for animals supplemented with high-starch without oil. In addition, oil supplementation decreased the numbers of Dasytricha ($P < 0.01$), Isotricha ($P < 0.01$), and total protozoa ($P < 0.01$). Rumen bacteria population were affected by starch × oil interaction. Therefore, the percentage of *Ruminococcus albus* ($P = 0.0003$), *Ruminococcus flavefaciens* ($P = 0.0002$), and *Archeas* ($P < 0.0004$) were higher for low-starch without oil diets than for other diets. Additionally,

animals supplemented with oil decreased the number of *Fibrobacter succinogenes* (P = 0.0003), independently of starch level used. Soybean hulls have a similar energy value to that of corn when supplemented to animals on grazing. Oil supplementation reduce intake, protozoa population, and fibrolytic rumen bacteria. The use of low-starch supplementation without oil may be effective to increase digestibility of DM and nutrient, and *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Archeas* population in the rumen of growing Nellore steers grazing tropical pasture.

Key words: lipids, microbes, protozoa, ruminant, soybean hulls

1. INTRODUCTION

The feed supplementation of beef cattle on grazing consists of an important technology to correct nutritional imbalances that may exist in the pasture. Several trials have studied the effect of concentrate type (Corrigan et. al., 2009; Schoonmaker et al., 2003; Patra and Yu, 2013), but very few analyzed this effect while dissociating the impact of energy intake from the type of energy source on rumen fermentation and microbial population of beef cattle.

Dietary effects, therefore, influence population dynamics, which need to be reliably and accurately determined by quantitative analysis of populations. This diversity feature, combined with an opportune supply of substrate and continuous removal of fermentation end products, leads to a high abundance of microbes, including cellulolytics, amylolytics, and lipolytics (Hobson and Stewart, 1997; Morgavi et al., 2013). Thus, intense and intricate interspecies interactions directly influence the performance of ruminant animals, including feed utilization efficiency, output of environmental pollutants (e.g., methane and ammonia), and host health (Firkins, 2010).

Supplemental non-fibrous carbohydrates such as grains can increase feed intake and provide additional energy to cattle grazing on tropical pastures. The conversion of carbohydrates to glucose and then pyruvate, the intermediate through which all carbohydrates must pass before being converted to volatile fatty acids (VFA), carbon dioxide and methane (Russell and Hespell, 1981). Therefore, the proportion of end products depends on type of carbohydrate fermented and also effects of pH and liquid dilution rates, which have a major influence on microbial populations and VFA production (Chalupa, 1997).

On the other hand, interest in use of supplemental fats to improve energy balance and productive efficiency by beef cattle led to intensive research on metabolism of lipids by ruminants (Chilliard, 1998). Effects of fats on ruminal fermentation of fiber has been a major focus, that stems from the demonstrated toxicity of long chain fatty acids, especially unsaturated fatty acids, to ruminal fibrolytic and methanogenic bacteria (Henderson,1973; Palmquist and Jenkins, 1980; Jenkins, 1993), which can result in changes microbial population, decrease fiber digestibility and DM intake.

Therefore, there have been few studies to date with beef cattle grazing pasture, which investigated the effect of starch-based supplementation level combined with oil on rumen microbial population and fermentation parameters. The hypothesis of the present study is that when combined with oil, soybean hulls could replace corn as a source of energy improving fermentation parameters and ruminal microbiota without affecting feed intake. This study evaluated the combined effects of high- or low-starch supplements and oil on intake, nutrient digestibility, rumen microbial population, and fermentation parameters of Nellore steers grazing *Brachiaria brizantha* cv. Xaraés in the rainy season, during the growing phase.

2. MATERIALS AND METHODS

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal) of the Faculty of Agriculture and Veterinary Sciences – São Paulo State University (UNESP) – Jaboticabal campus (protocol number 021119/11).

Animals and management

The The experiment was conducted at the UNESP (Jaboticabal, SP, Brazil) from December 2012 to May 2013, in the rainy season. Under the international Köppen classification this climate is characterized as tropical type AW with summer rains and relatively dry winter; the local altitude is 595 m, at 21°15'22" S, 48º18'58" W. The average maximum annual temperature is 29.1°C, and the average minimum annual temperature is 16.5°C. The average monthly precipitation is 105 mm, with 85% of the rainfall occurring between the months of October and March.

A replicate 4 × 4 Latin square experiment using eight ruminal cannulated Nellore steers (424.8 kg ± 35.5) at 20 mo of age were used to evaluate the combined effects of high- or low-starch supplements with or without a source of oil (2 steers per treatment) on intake, nutrient digestibility, ruminal pH, NH_3-N and volatile fatty acid concentration, and ruminal microbiology over four 21 d periods. Each period consisted of 14 d for adaptation to the supplement and 7 d for sampling.

Initially, the animals were weighted, identified, and treated against ecto- and endoparasites by administration of ivermectin 1% (Ivomec®, Merial, Paulínia, BR), and allocated into 4 paddocks of 0.25 ha each, consisting of *Brachiaria brizantha cv*. Xaraés. Pasture was formed in 2011. Fertilizer was applied only on once during entire the experimental period, 200 kg/ha of N:P2O5:K2O (20:05:20), at end of rainy season (May, 2013). The paddocks were fitted with smooth wire fencing, waterers and a pair of individually feed bunks.

The diets used consisted of two levels of starch-based supplement (corn or soybean hulls – SH) with or without a source of oil (ground soybean; GS). The supplements were corn combined with GS; corn without GS; SH combined with GS; and SH without GS. The crude glycerin was used in all supplements to replace (28% of DM) corn or SH. It is a byproduct from the biodiesel agroindustry and can be used in ruminant diets without compromising intake and performance (Drouillard, 2012; Parsons et al., 2009). Crude glycerin (83.90% glycerol, 1.75% ether extract [EE], 4.30% ash, and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil). The proportion of ingredients and chemical composition of supplements are presented in Table 1.

Animals were supplemented at the rate of 500 g/100 kg of BW, daily, at 1000 h, with 2 m of feed bunk arranged in each paddock, and had *ad libitum* access to water. Individual steers BW was recorded at the initiation of each period without fasting period, to adjust the amount of supplement.

Forage mass in each paddock was estimated in each period during the grazing study. The average sward height was taken by reading 50 sampling points in each paddock, using a graduated stick in cm (Barthram, 1985). Every 21 d, the average sward height, was utilized for sampling 4 sites, where all forage included within the perimeter of the rising plate (0.25 m^2) was collected by clipping at 5 cm above soil level from sites that represent the mean

forage mass of paddock. The clipping samples were dried to a constant weight under forced air at 55°C. Dry weights of these clippings were multiplied by the paddock area, to estimate the forage mass. Paddocks had an average forage mass of 10295.7 kg/ha ± 3439.2 and an average sward height of 39.0 cm ± 6.2. The grazing method used was the continuous grazing system (Allen et al., 2011), and the initial average sward height was 47.0 ± 5.9 cm. Forage samples were collected to be representative of diets consumed by grazing steers from all pastures in each period during the grazing studies by handling plucking methodology to mimic forage selected by grazing steers (Johnson, 1978). Handling plucking was performed on the same days as the estimation of DMI, described later. Samples were dried to a constant weight at 55°C under forced air.

Table 1. Experimental supplement and chemical composition of supplements and pasture (% DM basis)

Item	High Starch		Low Starch		Pasture[1]
	Oil	No Oil	Oil	No Oil	
Ingredient proportions					
Ground corn[*]	8.90	18.5	0.00	0.00	-
Soybean meal	0.00	49.0	0.00	49.0	-
Soybean hulls	0.00	0.00	8.50	18.5	-
Ground soybean[*]	58.6	0.00	59.0	0.00	-
Crude glycerin	28.0	28.0	28.0	28.0	-
Commercial premix[2]	4.50	4.50	4.50	4.50	-
Chemical composition					
Dry matter	90.9	88.1	90.2	88.2	-
Organic matter	91.7	89.5	90.9	89.2	92.7
Crude protein	27.6	26.5	26.2	26.0	15.9
NDF	13.2	11.0	17.5	20.2	61.2
Starch[3]	11.0	16.3	4.79	3.52	-
Ether extract	13.8	3.18	13.4	2.57	1.31
Gross energy, *Mcal/kg DM*	5.16	4.51	5.07	4.41	4.49

[1]Average and standard deviation of the mean of samples obtained by technique of simulated grazing in five periods.

[2]120 g Calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1,220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, and 300 mg fluorine.

[3]Calculated based on ingredient values from Valadares Filho et al., 2010.

[*]Ground in a hammermill fitted with screen size of 3.0 mm (fine).

Proximate analysis

For proximate analysis, the samples of ingredients, supplements, forage, and feces were dried at 55ºC for 72 h. Samples were then ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen, and analyzed for DM (method 934.01), OM (method 942.05), and EE (method 920.85) according to the Association of Official Analytical Chemists (AOAC, 1995). Concentrations of N in each sample were determined by rapid combustion (850°C), conversion of all N-combustion products to N_2, and subsequent measurement by thermoconductivity cell (Leco® model FP-528; LECO Corporation, St. Joseph, MI). Crude protein was calculated as the percentage of N in the sample multiplied by 6.25. The GE content of supplements, forage, and feces was determined using an adiabatic bomb calorimeter (model 6300; Parr Instrument Company, Moline, IL). Analyses for NDF were conducted following Van Soest et al. (1991) and adapted for the Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY). Heat-stable α-amylase was included in the NDF solution, without added sodium sulfite.

Intake estimation

Intake and nutrient digestibility were estimated in all of periods, using the marker method: lignin isolated, purified, and enriched from *Eucalyptus grandis* (LIPE®) and indigestible neutral detergent fiber (iNDF) were used to estimate the excretion of fecal matter (as dry weight), and forage intake, respectively. The intake of concentrate was obtained through the individual supply of supplement, calculated according to the body weight of the animal. Lignin isolated, purified, and enriched from *Eucalyptus grandis* was provided for 7 d by cannula infusion of a 500 mg bolus, with 4 d to stabilize fecal excretion of the marker, and in the last 3 d for sample collection (Saliba, 2005).

Fecal samples were collected on d 19, 20, and 21 of each period, directly from the rectum, at 1100 and 1700, 0900 and 1500, and 0700 and 1300 h, during the first, second, and third d of collection, respectively. The fecal samples were dried at 55°C for 72 h and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen and composited proportionately on each of 3 d and hours of sampling, within each animal, based on fecal dry weights. Approximately 10 g of each composited sample of feces was sent to the Federal University of Minas Gerais (Belo Horizonte, MG, Brazil) to estimate the total daily fecal output by LIPE® measurement methods as described by Saliba (2005).

The individual intake of forage was estimated using the internal marker iNDF. The samples of feces, forage, and concentrate were placed in Ankom bags (Filter bag F57; Ankom Technology Corporation) and incubated in the rumen of a cannulated Nellore animal for a period of 288 h (Valente et al., 2011). When the bags were withdrawn from the rumen, they were soaked in water for 30 min and gently washed by hand under running water until the wash water ran clear. The bags were then placed in an Ankom^{200} fiber Analyzer (Ankom Technology, Fairport, NY, USA), according to the methods described by Van Soest et al. (1991), and the iNDF was determined by weighing the bags with a digital scale after drying them in an oven, first at 55°C for 72 h and then at 105°C for 12 h. The residue was considered the iNDF. Individual forage intakes were estimated by subtracting marker excretion from the concentrate from the total iNDF excretion and dividing that difference by the concentration of the marker in the forage.

Ruminal fermentation

Rumen pH, ammonia N (NH_3-N), and volatile fatty acids (VFA) were measured for one day during the d 18 of each period. To assess rumen fermentation parameters, rumen fluid samples (around 80 mL) were collected manually at 0, 3, 6, 12 and 18 h after supplementation (1000 h). Rumen fluid was obtained from several sites within the rumen and was subsequently strained through two layers of cheesecloth. Immediately after collection, the pH of rumen fluid was determined using a digital potentiometer (ORION 710A, Boston, MA). An aliquot of collected fluid (50 mL each) was poured into 50 mL plastic bottle and frozen at -20°C for subsequent analysis of NH_3-N concentration. Ruminal fluid NH_3-N was analyzed by distilling with 2 M KOH in a micro-Kjeldahl system, according to the original procedures of Fenner (1965). The samples collected for analysis of VFA were centrifuged at 13.000 x g (4°C) for 30 min and quantified by gas chromatography (GC Shimatzu model 20-10, automatic injection) using capilar column (SP-2560, 100 m × 0.25 mm in diameter and 0.02 mm in thickness, Supelco, Bellefonte, PA) according to the methodology of Palmquist and Conrad (1971).

Rumen microbial profile

Ruminal microbiology (bacteria and protozoa) samples were collected on the d 18, after 3 h of supplementation (1000 h). For protozoa population cell counts were obtained from

rumen content aliquots that were preserved in formalin (a solution of equal parts water and 370 mL/L formaldehyde) according to D'Agosto and Carneiro, (1999). Ciliate protozoa species were identified and quantified the in chamber Sedgewick-Rafter, according to Dehority (1984). Each sample was homogenized and 1mL of ruminal content was pipetted and transferred to vials with lugol, according modified methodology from D'Agosto and Carneiro (1999). After 15 min, 9 mL of glycerin at 30% was added in vials. To quantify the protozoa, from each vails was pipetted 1mL of content to fill the chamber of Sedgewick-Rafter. The ciliates were measured according to Dehority (1984).

For the quantification and identification of rumen bacteria, fifty grams of the ruminal contents were weighed and immediately added to 50 mL of phosphate saline buffer (pH 7.4), stirred vigorously for 3 min, and then filtered with a mesh fabric (100 microns). The filtrate was subjected to centrifugation at 16,000 x g for 10 min at 4°C. The supernatant was discarded and the remaining precipitate was resuspended in 4 mL of Tris-EDTA buffer (10X, pH 8.0). The resuspended content was centrifuged at 16,000 x g for 10 min at 4°C, the supernatant was discarded, and the precipitate was immediately stored in refrigeration (-20°C) for a period of two months.

DNA extraction was conducted in 250 mg of sample using the extraction kit FastDNA® SPIN Kit for Soil (MP Biomedical, LLC). The integrity and quantity of the DNA was checked by electrophoresis on agarose gel (0.8%), and complementary DNA was assessed by spectrophotometry (Thermo Scientific NanoDrop™ 1000) for evaluation of its quality and quantity. For quantification of total bacteria and relative quantification of cellulolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefasciens, Anaerovibrio lipolytica, Selenomonas ruminantium,* and *Archeas*), the technique used was qPCR. The primers used in this study are shown in Table 2.

Three concentrations (400, 600, and 800 nM) of forward and reverse primers were tested to determine minimum primer concentration giving the lowest threshold cycle and to reduce nonspecific amplification before starting the reaction.

The amplifications were performed in triplicate and negative controls were run in the assay, omitting the total DNA. The reactions were conducted in the 7500 Real Time PCR System. Rox was used as a passive reference dye. The qPCR reaction was carried out using 100 ng of total DNA in a reaction containing: 7.5 μL of SYBR® Green PCR Master Mix (Bio-Rad, Hercules, California, USA), 10 pmol of primer pair, and H_2O to a final volume of

12.5 µL. Cycling conditions were 50°C for 2 min; 95°C for 10 min; and 40 cycles of 95°C for 15 seconds, 60°C for 1 min, and 78°C for 30 seconds. After amplification cycles, a step was added in which temperature was increased from 60 to 95°C to obtain dissociation curve of the reaction products, used for analyzing the specificity of amplification. Relative quantification was used to determine species proportion. The results were expressed as a 16S rDNA ratio of general bacteria, following the equation:

Relative quantification = $2^{-(Ct\ target - Ct\ total\ bacteria)}$;

Where C_t is defined as the number of cycles required for the fluorescent signal to cross the threshold.

Table 2. PCR primers used in this study for quantification of specific rumen microbes by qPCR

Primer	Sequence (5` to 3`)	Product size (bp)	Efficiency (%)
Total bacteria[*1]	F: CGGCAACGACAACCC R: CCATTGTAGCACCTGTGTAGCC	130	99
Fibrobacter succinogenes[1]	F: GGTATGGGATGAGCTTGC R: GCCTGCCCCTGAACTATC	121	98
Ruminococcus flavefasciens[1]	F: GGACGATAATGACGGTACTT R: GCAATC(CT)GAACTGGGACAAT	132	96
Ruminococcus albus[1]	F: CCCTAAAAGCAGTCTTAGTTCG R: CCTCCTTGCGGTTAGAACA	175	96
Total *Archeas*[2]	F: TTC GGT GGA TCD CAR AGR GC R: GBA RGT CGW AWC CGT AGA ATC C	140	95
Anaerovibrio lipolytica[4]	F: TTGGGTGTTAGAAATGGATTCTAGTG R: TCGAAATGT TGTCCCCAT CTG	82	98
Selenomonas ruminantium[3]	F: GGCGGGAAGGCAAGTCAGTC R: CCTCTCCTGCACTCAAGAAAGACAG	83	98

[*]Primers used for qPCR normalization; F = "forward"; R = "reverse".

[1]Denman and McSweeney. (2006).

[2]Denman et al. (2007).

[3]Khafipour et al. (2009).

[4]Fuentes et al. (2009).

Statistical Analysis

The experimental design was a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments (high or low starch, with or without a source of oil). Data of intake and apparent digestibility were analyzed considering a replicated Latin square design using the MIXED procedures (SAS Inst. Inc., Cary, NC, USA). The model included the fixed effect of treatment and Latin square, and random effects of period, animal, and error.

Repeated measures ANOVA was conducted using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) for a factorial analysis of time and treatments effects on data of pH, NH_3-N, and volatile fatty acids. The model included the fixed effect of treatment, time, and treatment × time interaction, and random effects of period, animal and error.

Data of protozoa were transformed to $\log^{10}$, plus a drive to meet the requirements of the SAS analysis. The analysis considered a replicated Latin square design using the MIXED procedures (SAS Inst. Inc., Cary, NC, USA). The model included the fixed effect of treatment and Latin square, and random effects of period, animal and error. Statistical analyses for bacteria population were performed using the MIXED procedures (SAS Inst. Inc., Cary, NC, USA). The model included the fixed effect of treatment, and random effects of period, animal and error.

Homogeneity of the data was verified using the UNIVARIATE procedure of SAS. Studentized residuals were plotted against the predicted values using the plot procedure to analyze data for outliers. The LSMEANS statement of the mixed procedure of SAS was used to calculate mean values. When the treatments were significant, the means were compared with Fisher's tests using the PDIFF option in LSMEANS command. The level of significance used to assess differences among means was $\alpha = 0.05$.

3. RESULTS

There were no interactions between starch level and oil supplementation with regard to intake of DM (% of BW, $P = 0.20$; kg/d, $P = 0.16$), forage DM ($P = 0.15$), supplement DM ($P = 0.66$), OM ($P = 0.12$), CP ($P = 0.08$), NDF ($P = 0.12$), EE ($P = 0.53$) and GE ($P = 0.20$). There were no effect of starch supplementation on intake of DM, forage DM, supplement DM, OM, CP, NDF, EE, and GE ($P > 0.05$). However, the addition of oil decreased the intake of DM ($P = 0.01$), forage DM ($P < 0.01$), OM ($P = 0.01$), CP ($P = 0.02$), NDF ($P < 0.01$), and GE ($P = 0.01$) independently of starch level used (Table 3).

Table 3. Effect of supplements containing high or low starch with or without oil (Oil or No Oil) on intake and total digestibility in Nellore steers on pasture during growing phase

Item	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Intake, % of BW								
DM	2.10	2.29	2.00	2.47	0.11	0.69	< 0.01	0.20
Intake, kg/d								
DM	8.88	9.53	8.57	10.60	0.54	0.43	0.01	0.16
Forage DM	6.78	7.43	6.42	8.46	0.52	0.48	< 0.01	0.15
Supplement DM	2.10	2.09	2.15	2.15	0.09	0.65	0.82	0.66
OM	8.20	8.77	7.91	10.04	0.52	0.32	0.01	0.12
CP	1.68	1.74	1.58	1.98	0.14	0.48	0.02	0.08
NDF	4.33	4.80	4.35	5.76	0.29	0.11	< 0.01	0.12
EE	0.36	0.18	0.35	0.18	0.02	0.75	< 0.01	0.53
GE, *Mcal/d*	40.93	43.83	39.78	48.57	2.51	0.43	0.01	0.20
Total digestibility, g/kg DM								
DM	717.81^{b}	711.85^{bc}	690.03^{c}	744.93^{a}	1.42	0.77	0.01	< 0.01
OM	738.71^{b}	733.02^{b}	718.77^{b}	775.72^{a}	1.28	0.31	0.01	< 0.01
CP	767.48^{ab}	745.47^{ab}	728.53^{b}	775.53^{a}	1.98	0.80	0.19	< 0.01
NDF	670.61^{b}	658.58^{b}	660.25^{b}	719.33^{a}	1.89	0.07	0.09	0.01
EE	794.23	633.86	731.60	669.02	6.48	0.79	0.04	0.36
GE	708.85^{ab}	707.68^{ab}	680.35^{b}	729.41^{a}	1.39	0.77	0.01	0.01

$^{a–c}$Means within a row with different superscripts differ ($P < 0.05$).

[1]High starch: 136 g/kg of starch in DM supplement.

[2]Low starch: 41.5 g/kg of starch in DM supplement.

Animals fed with low-starch and without oil had greater digestibility of DM ($P < 0.01$), OM ($P < 0.01$), CP ($P < 0.01$), NDF ($P = 0.01$), and GE ($P = 0.01$) than animals fed with other supplements. Consequently, the digestibility of NDF increased at 12.0% for animals supplemented with low-starch without oil (Table 3).

In relation to rumen fermentation parameters, there were no interactions between starch × oil for pH ($P = 0.30$), NH_3-N ($P = 0.77$), total VFA ($P = 0.73$), acetate ($P = 0.35$), propionate ($P = 0.76$), butyrate ($P = 0.06$), isobutyrate ($P = 0.47$), valerate ($P = 0.50$), isovalerate ($P = 0.51$), A:P ratio ($P = 0.54$). However, independently of starch level utilized, the addition of oil in the supplements decreased the pH ($P = 0.02$) and NH_3-N ($P = 0.02$). Furthermore, there was effect of time on pH, NH_3-N and VFA concentrations of Nellore on pasture during growing phase ($P < 0.01$; Table 4).

Table 4. Effects of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on pH, NH_3-N and volatile fatty acids concentrations in Nellore steers on pasture during growing phase

Item	High Starch		Low Starch			*P-value*					
	Oil	No Oil	Oil	No Oil	SEM	Starch	Oil	Starch × Oil	Time	Starch × Time	Oil × Time
pH	6.46	6.52	6.41	6.53	0.05	0.73	0.02	0.30	< 0.01	0.88	< 0.01
NH_3-N, mg/dL	12.54	14.08	12.38	13.62	1.31	0.59	0.02	0.77	< 0.01	0.02	< 0.01
VFA, mM											
Total VFA	78.64	78.25	78.16	78.96	8.35	0.96	0.93	0.73	< 0.01	0.53	0.01
Acetate	48.49	48.29	48.25	50.06	5.46	0.69	0.67	0.35	< 0.01	0.59	0.43
Propionate	15.55	15.09	15.52	14.84	1.97	0.88	0.37	0.76	< 0.01	0.57	< 0.01
Butyrate	10.90	11.27	11.05	10.53	0.92	0.65	0.84	0.06	< 0.01	0.81	0.03
Isobutyrate	0.97	1.05	0.91	0.94	0.06	0.01	0.16	0.47	< 0.01	0.98	< 0.01
Valerate	1.14	1.08	1.12	1.02	0.08	0.60	0.13	0.50	< 0.01	0.63	0.01
Isovalerate	1.46	1.57	1.40	1.44	0.12	0.27	0.33	0.51	< 0.01	0.73	< 0.01
A:P ratio[1]	3.41	3.58	3.36	3.59	0.42	0.89	0.09	0.54	< 0.01	0.48	0.92

[1]Acetate to propionate ratio.

There were no interactions between starch level and oil supplementation on numbers of Dasytricha (P = 0.89), Isotricha (P = 0.26), Polyplastron (P = 0.33), Ostracodinium (P = 0.26), and total protozoa (P = 0.93). However, there was interaction between starch and oil supplementation on Entodinium population (P = 0.04). Animals supplemented with high-starch and without oil showed greater numbers of Entodinium than the other supplements. In addition, independently of starch level utilized, oil supplementation decreased the numbers of Dasytricha (P < 0.01), Isotricha (P < 0.01), and total protozoa (P < 0.01; Table 5).

Table 5. Effect of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on rumen fluid protozoa numbers of Nellore steers on pasture during growing phase

Protozoa (n x 10^4 ml^{-1})[3]	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Entodinium	5.70[b]	6.01[a]	5.56[b]	5.60[b]	0.07	< 0.01	0.01	0.04
Dasytricha	4.27	4.87	4.18	4.81	0.13	0.64	< 0.01	0.89
Isotricha	3.79	4.06	3.47	4.01	0.17	0.30	< 0.01	0.26
Polyplastron	3.88	4.15	3.79	3.81	0.14	0.13	0.25	0.33
Ostracodinium	3.62	3.95	3.81	3.84	0.15	0.79	0.18	0.26
Total protozoa	18.50	22.08	17.66	21.12	0.93	0.26	< 0.01	0.93

[a–c]Means within a row with different superscripts differ (P < 0.05).

[1]High starch: 136 g/kg of starch in DM supplement.

[2]Low starch: 41.5 g/kg of starch in DM supplement.

[3]Log^{10} of number of protozoa.

Rumen bacteria population was affected by starch × oil interaction. Therefore, the percentage of *Ruminococcus albus* ($P = 0.0003$), *Ruminococcus flavefaciens* ($P = 0.0002$), and *Archeas* ($P < 0.0004$) was higher for low-starch without oil diets than for other diets. Additionally, animals supplemented with oil decreased the number of *Fibrobacter succinogenes* ($P = 0.0003$), independently of starch level used. On the other hand, high-starch supplementation increased the population of *Selenomonas ruminantium* ($P = 0.0276$; Table 6).

Table 6. Effect of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on relative proportion (%) of cellulolytic bacteria and methanogenic arqueas of Nellore steers on pasture during growing phase

Item	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Fibrobacter succinogenes	0.0677	0.0986	0.0652	0.0893	0.0052	0.3655	0.0003	0.4457
Ruminococcus albus	0.0028^{c}	0.0121^{b}	0.0034^{c}	0.0451^{a}	0.0024	0.0002	< 0.0001	0.0003
Ruminococcus flavefaciens	0.0002^{b}	0.0008^{b}	0.0015^{b}	0.0274^{a}	0.0017	< 0.0001	0.0001	0.0002
Anaerovibrio lipolytica	0.0177^{ab}	0.0151^{b}	0.0220^{a}	0.0014^{c}	0.0023	0.1246	< 0.0001	< 0.0001
Selenomonas ruminantium	0.0035	0.0022	0.0026	0.0006	0.0004	0.0276	0.0069	0.4434
Archeas	0.0830^{c}	0.2781^{b}	0.0167^{c}	0.8032^{a}	0.0515	0.0018	< 0.0001	0.0004

$^{a–c}$Means within a row with different superscripts differ ($P < 0.05$).

[1]High starch: 136 g/kg of starch in DM supplement.

[2]Low starch: 41.5 g/kg of starch in DM supplement.

4. DISCUSSION

This study evaluated the effects of oil supplementation combined with high- or low-starch on intake, digestibility, rumen fermentation, and rumen microbiota of Nellore steers fed *Brachiaria brizantha cv.* Xaraés during the growing phase. Interaction between starch × oil no affected intake, pH, NH_3-N, and VFA´s. However, animals fed with low-starch without oil increased digestibility of nutrient, percentage of *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Archeas* population. Moreover, independent of starch level used, the addition of oil to the diet was associated with decreased intake of nutrient, ruminal fermentation parameters (NH_3-N and ruminal pH), protozoa population, and fibrolytic bacterial population of rumen.

In relation to intake of DM and nutrients had no effect of interaction among high- or low-starch supplements and oil. These results are probably due to similarity between energy source (corn and SH) of supplements, that when combined with CP of pasture, resulted in synchronism between protein and energy in the rumen, increasing efficiencies of microbial protein synthesis in the rumen of animals on grazing pasture. In agreement with our data, McDonnell et al. (1982) evaluated increasing levels of soybean hulls and corn (0, 12.5, 25, or 50% of diet), by a 120 d period. Results within an energy level showed that DMI, ADG and F:G were not different when comparing corn and SH.

On the other hand, our results showed that the addition of ground soybean in diet (1.78% to 4.06% of EE) decreased the intake of DM (13.32%) and OM (14.4 %) of animals, independently of starch level used. This occurs because high levels of oil in diet of animals grazing pasture can adhere to the fiber component of feed, which produces a toxic effect on gram-positive bacteria (e.g. *Fibrobacter succinogenes*) by creating a physical barrier that impedes the activity of microorganisms and enzymes and consequently reduces feed intake and digestibility (Van Soest, 1994; McAllister et al., 1994; Palmquist and Jenkins, 1980; Sullivan et al., 2004). In agreement with previous studies, our data showed that animals fed with low-starch without oil increased the digestibility of DM (5.15%), OM (5.87%), and NDF (11.98%) in relation to animals fed with other supplements. Adding oil to the diet of growing beef cattle decreased feed intake by 21% and total tract digestibility of DM by 15% of that of control animals (Beauchemin and McGinn, 2006).

Rumen microbial population may be influenced by a wide range of variables such as diet chemical composition, ruminal pH, dietary forage:concentrate ratio, and ruminal NH_3

concentration (Allen and Mertens, 1987). Ruminal pH values for all treatments ranged between 6.41 and 6.53, which were within the range considered acceptable for fiber digestion (Ørskov and Ryle, 1990). Our findings show that there was no interaction between starch × oil for pH values, but the addition of oil in the supplements decreased rumen pH values of animals, this may be due to increased energy density of diet (Fulton et al., 1979) or the increased concentrations of total VFA (Baile and Forbes, 1974). Lipid digestion in the rumen favors the production of free short chain fatty acids and glycerol, which, in turn, is converted into propionate (Chalupa et al., 1986; Byers and Schelling, 1993).

Decreased of 10.00% in ruminal NH_3-N concentration occurred when animals were supplemented with oil, independently of starch level utilized. The concentration of N-NH_3 in the rumen results from the balance between production, absorption and incorporation of amino N into microbial protein (Russell et al., 1983). In our study, ruminal NH_3-N concentrations ranged between 12.38 to 14.08 mg/dL. These values were similar to the concentration suggested by Detmann et al. (2009) to maximize fiber intake (15 mg/dL), but still lower than the value described by Leng (1990) to maximize voluntary intake (20 mg/dL). Reduction of NH_3-N concentrations with oil supplementation reflects increased ammonia consumed by ruminal bacteria, which was considered as an indicator of increased of microbial protein synthesis (Kholif et al., 2014).

Regarding volatile fatty acid, our findings showed no interaction between starch × oil for concentrations of acids. Of the VFA evaluated, propionate would have been expected to increase with high-starch supplements because fermentation of starch and sugars promotes an increased production of propionate (Van Soest, 1994). However, when animals were fed with low-starch supplements without oil, there was an increase of organic matter and fiber digestibility, which ultimately leads to an increase of VFA production and, thus, had no differences between starch × oil for concentrations of acids. The higher concentrations of total VFA indicate the more efficient anaerobic fermentation, which may be due to increased organic matter and fibers digestibility (Khattab et al., 2011). Total VFA concentration in the rumen depends on many factors including nutrient digestibility, rate of absorption, rumen pH, rate of digesta passage from rumen, as well as the microbial population in the rumen and their activities (Flatt et al., 1956).

Rumen protozoa interact with other microbes in the rumen (i.e., bacteria, anaerobic fungi, and methanogens) and can modify the structure of the rumen microbial ecosystem

(Yáñez-Ruiz et al., 2006; Mosoni et al., 2011). However, there is little evidence as to whether these changes are due to protozoal predatory activity (Newbold et al., 1989) or protozoa-substrate interactions, in which case, it would be modulated by the diet.

Our results show that animals supplemented with high-starch and without oil showed greater numbers of Entodinium than the other supplements. This genre besides being the most representative of ciliated protozoa in ruminants, it has a great one ability to adapt to diets with high inclusion of starch (Hook et al. 2011). Ciliate protozoa play a vital role in rumen fermentation, once that exert a protection of easily fermentable carbohydrates (sugars and starch) from sugar-/starch-utilizing bacteria (Kamra, 2005) so that organic acids are not produced in plenty immediately after the feeding of animals. However, these sugars are released slowly during the day so that there is a constant supply of energy for the animals in the form of short-chain volatile fatty acids.

On the other hand, independently of starch level utilized, oil supplementation decreased the numbers of Dasytricha, Isotricha, and total protozoa (Beauchemin et al., 2008; Ivan et al., 2004). These results indicate a toxic effect of lipids (e.g. ground soybean) on these genres of protozoa, and agree with studies of Hristov et al. (2004) who reported that protozoa are sensitive to unsaturated fatty acids C18:3, C18:2, and C18:1.

In relation to rumen bacteria population, the percentage of *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Archeas* was higher for low-starch without oil diets than for other diets. This explains the results already presented in our study in relation to the greater values of DM (5.15%), OM (5.87%), and NDF (11.98%) digestibility than animals fed with other supplements. These interactions may be due to inhibiting effect of supplementation on the ruminal microbial population, directly by toxins or indirectly by reduction of nutrient substrate availability for the microbes. Biohydrogenation of PUFA is not likely a major sink for reducing equivalents produced during fermentation, so BH is primarily a defense against PUFA toxicity (Jenkins et al., 2008). This toxicity is generally considered more severe with increasing intake of starch but not sugar under moderate inclusion rates (Martel et al., 2011). Toxicity of PUFA is more likely a result of metabolic interruption (depressed intracellular ATP and acyl CoA pools) rather than membrane toxicity (Maia et al., 2010).

Additionally, animals supplemented with oil decreased the number of *Fibrobacter succinogenes*, independently of starch level used. These results may explain the negative effects of lipid supplementation on digestibility and nutrients. *Fibrobacter succinogenes* (one

of the most prevalent cellulolytic bacteria) encodes for several enzymes capable of degrading an array of polysaccharides (Suen et al. 2011). This species appears to use these enzymes to gain access to cellulose in plant particles by solubilizing the compounds surrounding cellulose fibers, but appears to utilise only glucose, cellobiose and cellodextrins to obtain energy for maintenance and growth.

The proportion of *Anaerovibrio lipolytica* for animals fed with oil supplementation, independently of starch-based, was 12 and 15 times higher than diet with low-starch without oil, respectively. Due to use of soybean grains, the growth these gram-positive microorganisms was stimulated, once, they have the capacity to hydrolyze triglycerides into glycerol and fatty acids (Harfoot and Hazlewood, 1997). Long-chain fatty acids are directly toxic to methanogens, protozoa and gram-positive cellulolytic bacteria (Desbois and Smith, 2010; Nagaraja et al., 1997; Zeitz et al., 2013), which accounts for the depressed fiber digestion and reduced ruminal acetate and butyrate production associated with diets containing high concentrations of fatty acids (Van Nevel, 1991). Gram-negative, propionate-producing bacteria, however, are not significantly inhibited by fatty acids (Van Nevel and Demeyer, 1988).

Concerning the total population of Archeas, there was interation between starch × oil supplements. Nonetheless, the effect more pronounced was for low-starch supplements without oil, since, the population of Archeas was increased at 84.32% when compared with other supplements. These results are due to inhibitory effect presented by lipids on protozoa. Archeas works symbiotically with protozoa participating in transfers of hydrogen, which is used to reduce CO_2 to CH_4 (Newbold et al., 1995). The oil can also reduce the concentration of hydrogen in the rumen by means of biohydrogenation, acting as sink of hydrogen and reducing the activity of Archeas (Czerkawski, 1972).

5. CONCLUSION

Soybean hulls have a similar energy value comparable to that of corn when supplemented to animals on grazing, as indicated by the similar intake, digestibility, and rumen fermentation parameters of these sources of energy. Oil supplementation reduces intake, pH, NH_3-N, population of Dasytricha, Isotricha, and total protozoa, and number of *Fibrobacter succinogenes* in the rumen. The use of low-starch supplementation without oil may be effective to increase digestibility of DM and nutrient, and *Ruminococcus albus*,

Ruminococcus flavefaciens, and *Archeas* population in the rumen of growing Nellore steers grazing *Brachiaria brizantha* cv. Xaraés during the rainy season.

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CHAPTER 6

O artigo a seguir está redigido conforme normas de publicação do *Journal of Animal Science* exceto o posicionamento das tabelas.

RUMEN MICROBIAL POPULATION AND FERMENTATION PARAMETERS OF NELLORE STEERS FED WITH TWO LEVELS OF STARCH-BASED SUPPLEMENT WITH OR WITHOUT OIL ON FINISHING PHASE

ABSTRACT: The objective of this study was evaluate the effects of two levels of starch-based supplement combined with or without oil on intake, digestibility, rumen microbial population, and fermentation parameters of Nellore steers grazing *Brachiaria brizantha cv.* Xaraés during the finishing phase. Eight ruminal cannulated Nellore steers (514.5 kg ± 30.1) at 24 mo of age were used in a replicate 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments (high or low starch, with or without a source of oil) and an experimental period of 21 d. The diets used consisted of two levels of starch-based supplement (corn or soybean hulls – SH) with or without a source of oil (ground soybean; GS). The supplements were corn combined with GS; corn without GS; SH combined with GS; and SH without GS. Animals were supplemented at the rate of 1000 g/100 kg BW. There were no interactions between starch-based supplementation level and oil with regard to intake of DM (% of BW, $P = 0.60$; kg/d, $P = 0.70$), forage DM ($P = 0.63$), supplement DM ($P = 0.50$), OM ($P = 0.67$), CP ($P = 0.42$), NDF ($P = 0.38$), EE ($P = 0.20$) and GE ($P = 0.62$). Animals supplemented with low starch and no oil showed greater (10.77%) digestibility of CP ($P = 0.01$) than those supplemented with high starch and no oil. Total apparent digestibility of DM ($P < 0.01$), OM ($P < 0.01$), NDF ($P = 0.03$), and GE ($P = 0.02$) decreased with oil supplementation, independent of starch level used. There were no interactions between starch × oil for pH ($P = 0.39$), NH_3-N ($P = 0.47$), and total VFA ($P = 0.44$). Animals supplemented with oil showed lower acetate production ($P < 0.01$) than those supplemented without oil, independent of starch level. There was main effect of starch supplementation on numbers of Entodinium ($P < 0.01$), and total protozoa ($P < 0.01$). Furthermore, independently of starch level utilized, the addition of oil in the supplements decreased the population of Dasytricha ($P < 0.01$), Polyplastron ($P < 0.21$), and Diploplastron ($P = 0.04$). A significant interaction between starch level and oil supplementation was observed for *Ruminococcus albus* ($P = 0.0120$). Supplementing the animals with low-starch without oil increased the numbers of *Ruminococcus albus* compared with the other supplements. There was also interaction between starch × oil for *Selenomonas ruminantium* ($P = 0.0003$), once, low-starch supplement, with or without oil, decreased the number of *Selenomonas ruminantium* of Nellore steers. The addition of oil in the supplements decreased the number of *Fibrobacter*

succinogenes ($P < 0.0001$), *Ruminococcus flavefasciens* ($P < 0.0001$), and *Archeas* ($P < 0.0001$), but increased of *Anaerovibrio lipolytica* ($P < 0.0001$), independently of starch level used. Oil supplementation decreases intake, digestibility, acetate production, protozoa population, and fibrolytic rumen bacteria. The use of soybean hulls without oil supplementation may be effective to increase digestibility of CP, and *Ruminococcus albus* of finishing Nellore steers grazing *Brachiaria brizantha* cv. Xaraés during the dry season.
Key words: ammonia, lipids, ruminant, soybean hulls

1. INTRODUCTION

Concentrate supplements are included in diet of animals grazing pasture for correct an unbalance between protein and energy, and associative effects may occur if digestive and metabolic interactions between them decreases the efficiency of the utilization of metabolizable energy and limits intake (Poppi and McLennan, 1995; Dixon and Stockdale, 1999).

Ammonia is the preferred nitrogen source of fibrolytic bacteria in the rumen, and provision of ruminal degradable fiber (e.g. pectin) may stabilize ruminal pH and increase ruminal ammonia utilization (Russell et al., 1992; Firkins, 1997). Fermentation rate is an inherent property of feed, and the amount of feed energy per unit of time is positively associated with microbial efficiency (Van Soest, 1994; Russell et al., 1992). Thus, the rate of absorption of ammonia by ruminants suggests that energy availability, or lack of synchrony between energy and nitrogen supplies, limits the use of available nitrogen by ruminal microorganisms (Huntington, 1997).

Supplementation with soybean grains in finishing beef cattle diet is an interesting nutritional management to increase energy density (Zinn and Jorquera, 2007; Hess et al., 2008). Inclusion of lipid in finishing diets reduces ruminal OM digestibility but shifts the site of digestion to the lower parts of the gastrointestinal tract (Zinn and Shen, 1996; Plascencia et al., 2003). Consequently, short chain fatty acid production in the rumen can be reduced, thereby reducing the risk of metabolic disorders, and improve ruminal fermentation parameters (Aschenbach et al., 2011). Moreover, a decrease in protozoa population, which are predatory on ruminal bacteria, may occur when lipids are supplemented, thus increasing efficiency of bacterial growth (Ushida et al., 1984; Kayouli et al., 1986).

Therefore, there have been few studies to date that investigated the effect of starch-based supplementation level combined with oil on rumen microbial population and fermentation parameters of beef cattle. The hypothesis of the present study is that when combined with oil, SH could replace corn as a source of energy and could improve fermentation parameters and ruminal microbiota without affecting feed intake. This study evaluated the effects of two levels of starch-based supplement with or without oil on intake, digestibility, rumen microbial population, and fermentation parameters of Nellore steers grazing *Brachiaria brizantha cv.* Xaraés in the dry season, during the finishing phase.

2. MATERIALS AND METHODS

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal) of the Faculty of Agriculture and Veterinary Sciences – São Paulo State University (UNESP) – Jaboticabal campus (protocol number 021119/11).

Animals and management

The experiment was conducted at the UNESP (Jaboticabal, SP, Brazil) from May to July 2013, in the dry season. Under the international Köppen classification this climate is characterized as tropical type AW with summer rains and relatively dry winter; the local altitude is 595 m, at 21°15'22" S, 48º18'58" W. The average maximum annual temperature is 29.1°C, and the average minimum annual temperature is 16.5°C. The average annual precipitation is 105 mm, with 85% of the rainfall occurring between the months of October and March.

A replicate 4 × 4 Latin square experiment using eight ruminal cannulated Nellore steers (514.5 kg ± 30.1) at 24 mo of age were used to evaluate the combined effects of high- or low-starch supplements and oil (2 steers per treatment) on intake, nutrient digestibility, ruminal pH, NH_3-N and volatile fatty acid concentration, and ruminal microbiology over four 21 d periods. Each period consisted of 14 d for adaptation to the supplement and 7 d for sampling.

Initially, the animals were weighted, identified, and treated against ecto- and endoparasites by administration of ivermectin 1% (Ivomec®, Merial, Paulínia, BR), and

allocated into 4 paddocks of 0.25 ha each, consisting of *Brachiaria brizantha* cv. Xaraés. Pasture was formed in 2011. Fertilizer was applied only on once during entire the experimental period, 200 kg/ha of $N:P_2O_5:K_2O$ (20:05:20), at end of rainy season (May, 2013). The paddocks were fitted with smooth wire fencing, waterers and a pair of individually feed bunks.

The diets used consisted of two levels of starch-based supplement (corn or soybean hulls – SH) with or without a source of oil (ground soybean; GS). The supplements were corn combined with GS; corn without GS; SH combined with GS; and SH without GS. Crude glycerin was used in all supplements to replace (28% of DM) corn or SH. Crude glycerin is a byproduct from the biodiesel agroindustry and can be used in ruminant diets without compromising intake and performance (Drouillard, 2012; Parsons et al., 2009). Crude glycerin (83.90% glycerol, 1.75% ether extract [**EE**], 4.30% ash, and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil). The proportion of ingredients and chemical composition of supplements are presented in Table 1.

Animals were supplemented at the rate of 1000 g/100 kg of BW, daily, at 1000 h, with 2 m of feed bunk arranged in each paddock, and had *ad libitum* access to water. Individual steers BW was recorded at the initiation of each period without fasting period, to adjust the amount of supplement.

Forage mass in each paddock was estimated in each period during the grazing study. The average sward height was taken by reading 50 sampling points in each paddock, using a graduated stick in cm (Barthram, 1985). Every 21 d, the average sward height, was utilized for sampling 4 sites, where all forage included within the perimeter of the rising plate (0.25 m^2) was collected by clipping at 5 cm above soil level from sites that represent the mean forage mass of paddock. The clipping samples were dried to a constant weight under forced air at 55°C. Dry weights of these clippings were multiplied by the paddock area, to estimate the forage mass. Paddocks had an average forage mass of 8350.9 kg/ha ± 865.7 and an average sward height of 32.0 cm ± 5.7. The grazing method used was the continuous grazing system (Allen et al., 2011), and the initial average sward height was 39.0 ± 4.6 cm. Forage samples were collected to be representative of diets consumed by grazing steers from all pastures in each period during the grazing studies by handling plucking methodology to mimic forage selected by grazing steers (Johnson, 1978). Handling plucking was performed

on the same days as the estimation of DMI, described later. Samples were dried to a constant weight at 55°C under forced air.

Table 1. The ingredient proportions and chemical composition of supplements and pasture (% DM basis)

Item	High Starch		Low Starch		Pasture[1]
	Oil	No Oil	Oil	No Oil	
Ingredient proportions					
Ground corn[*]	18.5	31.0	0.00	0.00	-
Soybean meal	0.00	38.5	0.00	37.0	-
Soybean hulls	0.00	0.00	18.5	32.5	-
Ground soybean[*]	51.0	0.00	51.0	0.00	-
Crude glycerin	28.0	28.0	28.0	28.0	-
Commercial premix[2]	2.50	2.50	2.50	2.50	-
Chemical composition					
Dry matter	90.2	89.3	90.3	89.4	-
Organic matter	92.3	92.2	91.7	91.3	92.6
Crude protein	22.9	22.3	23.9	23.6	12.1
NDF	12.7	11.1	21.9	27.1	60.4
Starch[3]	17.2	24.7	4.45	3.29	-
Ether extract	12.4	3.62	11.8	2.58	2.16
Gross energy, *Mcal/kg DM*	5.08	4.62	4.98	4.45	4.53

[1]Average and standard deviation of the mean of samples obtained by technique of simulated grazing in five periods.
[2]120 g Calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1,220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, and 300 mg fluorine.
[3]Calculated based on ingredient values from Valadares Filho et al., 2010.
[*]Ground in a hammermill fitted with screen size of 3.0 mm (fine).

Proximate analysis

For proximate analysis, the samples of ingredients, supplements, forage, and feces were dried at 55ºC for 72 h. Samples were then ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen, and analyzed for DM (method 934.01), OM (method 942.05), and EE (method 920.85) according to the Association

of Official Analytical Chemists (AOAC, 1995). Concentrations of N in each sample were determined by rapid combustion (850°C), conversion of all N-combustion products to N_2, and subsequent measurement by thermo conductivity cell (Leco® model FP-528; LECO Corporation, St. Joseph, MI). Crude protein was calculated as the percentage of N in the sample multiplied by 6.25. The GE content of supplements, forage, and feces was determined using an adiabatic bomb calorimeter (model 6300; Parr Instrument Company, Moline, IL). The NDF contents were analyzed using the Ankom^{200} Fiber Analyzer (Ankom Technology, Fairport, NY, USA) according to the methods described by Van Soest et al. (1991). Heat-stable α-amylase was included in the NDF solution, without added sodium sulfite.

Intake estimation

Intake and nutrient digestibility were estimated in all of periods, using the marker method: lignin isolated, purified, and enriched from *Eucalyptus grandis* (LIPE®) and indigestible neutral detergent fiber (iNDF) were used to estimate the excretion of fecal matter (as dry weight), and forage intake, respectively. The intake of concentrate was obtained through the individual supply of supplement, calculated according to the body weight of the animal. Lignin isolated, purified, and enriched from *Eucalyptus grandis* was provided for 7 d by cannula infusion of a 500 mg bolus, with 4 d to stabilize fecal excretion of the marker, and in the last 3 d for sample collection (Saliba, 2005).

Fecal samples were collected on d 19, 20, and 21 of each period, directly from the rectum, at 1100 and 1700, 0900 and 1500, and 0700 and 1300 h, during the first, second, and third d of collection, respectively. The fecal samples were dried at 55°C for 72 h and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen and composited proportionately on each of 3 d and hours of sampling, within each animal, based on fecal dry weights. Approximately 10 g of each composited sample of feces was sent to the Federal University of Minas Gerais (Belo Horizonte, MG, Brazil) to estimate the total daily fecal output by LIPE® measurement methods as described by Saliba (2005).

The individual intake of forage was estimated using the internal marker iNDF. The samples of feces, forage, and concentrate were placed in Ankom bags (Filter bag F57; Ankom Technology Corporation) and incubated in the rumen of a cannulated Nellore animal for a period of 288 h (Valente et al., 2011). When the bags were withdrawn from the rumen, they were soaked in water for 30 min and gently washed by hand under running water until the

wash water ran clear. The bags were then placed in an Ankom^{200} fiber Analyzer (Ankom Technology, Fairport, NY, USA), according to the methods described by Van Soest et al. (1991), and the iNDF was determined by weighing the bags with a digital scale after drying them in an oven, first at 55°C for 72 h and then at 105°C for 12 h. The residue was considered the iNDF. Individual forage intakes were estimated by subtracting marker excretion from the concentrate from the total iNDF excretion and dividing that difference by the concentration of the marker in the forage.

Ruminal fermentation

Rumen pH, ammonia N (NH_3-N), and volatile fatty acids (VFA) were measured for one day during the d 18 of each period. To assess rumen fermentation parameters, rumen fluid samples (around 80 mL) were collected manually at 0, 3, 6, 12 and 18 h after supplementation (1000 h). Rumen fluid was obtained from several sites within the rumen and was subsequently strained through two layers of cheesecloth. Immediately after collection, the pH of rumen fluid was determined using a digital potentiometer (ORION 710A, Boston, MA). An aliquot of collected fluid (50 mL each) was poured into 50 mL plastic bottle and frozen at -20°C for subsequent analysis of NH_3-N concentration. Ruminal fluid NH_3-N was analyzed by distilling with 2 M KOH in a micro-Kjeldahl system, according to the original procedures of Fenner (1965). The samples collected for analysis of VFA were centrifuged at 13.000 x g (4°C) for 30 min and quantified by gas chromatography (GC Shimatzu model 20-10, automatic injection) using capilar column (SP-2560, 100 m × 0.25 mm in diameter and 0.02 mm in thickness, Supelco, Bellefonte, PA) according to the methodology of Palmquist and Conrad (1971).

Rumen microbial profile

Ruminal microbiology (bacteria and protozoa) samples were collected on the d 18, after 3 h of supplementation (1000 h). For protozoa population cell counts were obtained from rumen content aliquots that were preserved in formalin (a solution of equal parts water and 370 mL/L formaldehyde) according to D'Agosto & Carneiro, (1999). Ciliate protozoa species were identified and quantified the in chamber Sedgewick-Rafter, according to Dehority (1984). Each sample was homogenized and 1mL of ruminal content was pipetted and transferred to vials with lugol, according modified methodology from

D' Agosto & Carneiro (1999). After 15 min, 9 mL of glycerin at 30% was added in vials. To quantify the protozoa, from each vails was pipetted 1mL of content to fill the chamber of Sedgewick-Rafter. The ciliates were measured according to Dehority (1984).

For the quantification and identification of rumen bacteria, fifty grams of the ruminal contents were weighed and immediately added to 50 mL of phosphate saline buffer (pH 7.4), stirred vigorously for 3 min, and then filtered with a mesh fabric (100 microns). The filtrate was subjected to centrifugation at 16,000 x g for 10 min at 4°C. The supernatant was discarded and the remaining precipitate was resuspended in 4 mL of Tris-EDTA buffer (10X, pH 8.0). The resuspended content was centrifuged at 16,000 x g for 10 min at 4°C, the supernatant was discarded, and the precipitate was immediately stored in refrigeration (-20°C) for a period of two months.

DNA extraction was conducted in 250 mg of sample using the extraction kit FastDNA® SPIN Kit for Soil (MP Biomedical, LLC). The integrity and quantity of the DNA was checked by electrophoresis on agarose gel (0.8%), and complementary DNA was assessed by spectrophotometry (Thermo Scientific NanoDrop™ 1000) for evaluation of its quality and quantity.

For quantification of total bacteria and relative quantification of cellulolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefasciens, Anaerovibrio lipolytica, Selenomonas ruminantium,* and *Archeas*), the technique used was qPCR. The primers used in this study are shown in Table 2.

Three concentrations (400, 600, and 800 nM) of forward and reverse primers were tested to determine minimum primer concentration giving the lowest threshold cycle and to reduce nonspecific amplification before starting the reaction.

The amplifications were performed in triplicate and negative controls were run in the assay, omitting the total DNA. The reactions were conducted in the 7500 Real Time PCR System. Rox was used as a passive reference dye. The qPCR reaction was carried out using 100 ng of total DNA in a reaction containing: 7.5 µL of SYBR® Green PCR Master Mix (Bio-Rad, Hercules, California, USA), 10 pmol of primer pair, and H_2O to a final volume of 12.5 µL. Cycling conditions were 50°C for 2 min; 95°C for 10 min; and 40 cycles of 95°C for 15 seconds, 60°C for 1 min, and 78°C for 30 seconds. After amplification cycles, a step was added in which temperature was increased from 60 to 95°C to obtain dissociation curve of the reaction products, used for analyzing the specificity of amplification.

Table 2. PCR primers used in this study for quantification of specific rumen microbes by qPCR

Primer	Sequence (5` to 3`)	Product size (bp)	Efficiency (%)
Total bacteria[*1]	F: CGGCAACGACAACCC R: CCATTGTAGCACCTGTGTAGCC	130	99
Fibrobacter succinogenes[1]	F: GGTATGGGATGAGCTTGC R: GCCTGCCCCTGAACTATC	121	98
Ruminococcus flavefasciens[1]	F: GGACGATAATGACGGTACTT R: GCAATC(CT)GAACTGGGACAAT	132	96
Ruminococcus albus[1]	F: CCCTAAAAGCAGTCTTAGTTCG R: CCTCCTTGCGGTTAGAACA	175	96
Total *Archeas*[2]	F: TTC GGT GGA TCD CAR AGR GC R: GBA RGT CGW AWC CGT AGA ATC C	140	95
Anaerovibrio lipolytica[4]	F: TTGGGTGTTAGAAATGGATTCTAGTG R: TCGAAATGT TGTCCCCAT CTG	82	98
Selenomonas ruminantium[3]	F: GGCGGGAAGGCAAGTCAGTC R: CCTCTCCTGCACTCAAGAAAGACAG	83	98

[*]Primers used for qPCR normalization; F = "forward"; R = "reverse".

[1]Denman and McSweeney. (2006).

[2]Denman et al. (2007).

[3]Khafipour et al. (2009).

[4]Fuentes et al. (2009).

Relative quantification was used to determine species proportion. The results were expressed as a 16S rDNA ratio of general bacteria, following the equation:

Relative quantification = $2^{-(Ct\ target - Ct\ total\ bacteria)}$;

Where C_t is defined as the number of cycles required for the fluorescent signal to cross the threshold.

Statistical Analysis

The experimental design was a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments (high or low starch, with or without a source of oil). Data of intake and apparent digestibility were analyzed considering a replicated Latin square design using

the MIXED procedures (SAS Inst. Inc., Cary, NC, USA). The model included the fixed effect of treatment and Latin square, and random effects of period, animal, and error.

Repeated measures ANOVA was conducted using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) for a factorial analysis of time and treatments effects on data of pH, NH_3-N, and VFA. The model included the fixed effect of treatment, time and treatment x time interaction, and random effects of period, animal and error.

Data of protozoa were transformed to $\log^{10}$, plus a drive to meet the requirements of the SAS analysis. The analysis considered a replicated Latin square design using the MIXED procedures (SAS Inst. Inc., Cary, NC, USA). The model included the fixed effect of treatment and Latin square, and random effects of period, animal and error. Statistical analyses for bacteria population were performed using the MIXED procedures (SAS Inst. Inc., Cary, NC, USA). The model included the fixed effect of treatment, and random effects of period, animal and error. Homogeneity of the data was verified using the UNIVARIATE procedure of SAS. Studentized residuals were plotted against the predicted values using the plot procedure to analyze data for outliers. The LSMEANS statement of the mixed procedure of SAS was used to calculate mean values. When the treatments were significant, the means were compared with Fisher's tests using the PDIFF option in LSMEANS command. The level of significance used to assess differences among means was $\alpha = 0.05$.

3. RESULTS

There were no interactions between starch-based supplementation level and oil with regard to intake of DM (% of BW, $P = 0.60$; kg/d, $P = 0.70$), forage DM ($P = 0.63$), supplement DM ($P = 0.50$), OM ($P = 0.67$), CP ($P = 0.42$), NDF ($P = 0.38$), EE ($P = 0.20$) and GE ($P = 0.62$). However, there was main effect of starch on intake of NDF ($P < 0.01$) and EE ($P < 0.01$). The addition of oil decreased the intake of DM ($P < 0.01$), forage DM ($P = 0.01$), OM ($P = 0.01$), and NDF ($P < 0.01$) independently of starch level used (Table 3).

There were no interactions between starch level and oil supplementation on digestibility of DM ($P = 0.27$), OM ($P = 0.16$), NDF ($P = 0.32$), EE ($P = 0.13$), and GE ($P = 0.27$). However, there was an interaction between starch and oil supplementation on CP digestibility ($P = 0.01$). Thus, animals supplemented with low-starch and no oil showed greater (10.77%) digestibility of CP than those supplemented with high-starch and no oil. The digestibility of DM ($P < 0.01$), OM ($P < 0.01$), NDF ($P = 0.03$), and GE ($P = 0.02$) decreased with oil supplementation, but digestibility of EE ($P < 0.01$) increased with the inclusion of the oil source, independent of starch level (Table 3).

Table 3. Effect of supplements containing high or low starch with or without oil (Oil or No Oil) on intake and digestibility in Nellore steers on pasture during finishing phase

Item	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Intake, % of BW								
DM	2.37	2.50	2.39	2.47	0.14	0.84	0.04	0.60
Intake, kg/d								
DM	12.17	12.98	12.11	12.74	0.59	0.54	< 0.01	0.70
Forage DM	7.04	7.81	7.02	7.55	0.65	0.57	0.01	0.63
Supplement DM	5.12	5.17	5.09	5.18	0.12	0.76	0.05	0.50
OM	11.24	11.99	11.17	11.72	0.55	0.45	0.01	0.67
CP	2.08	2.20	2.18	2.23	0.23	0.15	0.05	0.42
NDF	4.89	5.24	5.31	5.91	0.28	< 0.01	< 0.01	0.38
EE	0.78	0.35	0.76	0.29	0.02	< 0.01	< 0.01	0.20
GE, *Mcal/d*	58.04	59.36	57.25	57.47	2.89	0.24	0.49	0.62
Digestibility, g/kg DM								
DM	623.32	656.50	618.76	665.13	1.99	0.73	< 0.01	0.27
OM	647.75	679.23	641.42	689.97	1.88	0.71	< 0.01	0.16
CP	577.23^{bc}	559.42^{c}	591.75^{b}	627.00^{a}	4.80	< 0.01	0.40	0.01
NDF	518.80	534.88	542.03	583.27	2.99	< 0.01	0.03	0.32
EE	708.25	545.11	686.90	611.05	2.72	0.43	< 0.01	0.13
GE	603.27	632.23	612.00	622.92	2.13	0.97	0.02	0.27

$^{a–c}$Means within a row with different superscripts differ ($P < 0.05$).

[1]High starch: 209 g/kg of starch in DM supplement.

[2]Low starch: 38 g/kg of starch in DM supplement.

There were no interactions between starch × oil for pH (P = 0.39), NH_3-N (P = 0.47), total VFA (P = 0.44), acetate (P = 0.95), propionate (P = 0.88), butyrate (P = 0.92), isobutyrate (P = 0.42), valerate (P = 0.79), isovalerate (P = 0.86), A:P ratio (P = 0.08). However, animals supplemented with oil showed lower (9.25%, $P < 0.01$) production of acetate than those supplemented without oil. Furthermore, there was effect of time on pH, NH_3-N and VFA concentrations of Nellore steers on pasture during finishing phase ($P < 0.01$; Table 4).

Table 4. Effects of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on pH, NH_3-N and volatile fatty acids concentrations of Nellore steers on pasture during finishing phase

Item	High Starch[1]		Low Starch[2]			*P-value*					
	Oil	No Oil	Oil	No Oil	SEM	Starch	Oil	Starch × Oil	Time	Starch × Time	Oil × Time
pH	6.48	6.49	6.43	6.49	0.05	0.53	0.44	0.39	< 0.01	0.02	0.71
NH_3-N, mg/dL	19.46	20.35	20.71	20.23	1.37	0.64	0.86	0.47	0.16	0.36	0.73
VFA, mM											
Total VFA	84.16	89.26	87.53	89.01	5.73	0.58	0.24	0.44	< 0.01	< 0.01	0.54
Acetate	41.53	45.80	43.61	48.02	1.85	0.19	< 0.01	0.95	< 0.01	< 0.01	0.06
Propionate	20.58	21.89	21.83	22.97	2.06	0.57	0.55	0.88	< 0.01	0.04	0.92
Butyrate	17.65	15.67	15.90	13.80	1.40	0.17	0.12	0.92	< 0.01	0.13	0.07
Isobutyrate	1.10	1.22	1.09	1.12	0.09	0.45	0.32	0.42	< 0.01	0.50	0.41
Valerate	1.95	1.95	1.88	1.90	0.13	0.57	0.94	0.79	< 0.01	0.18	0.73
Isovalerate	1.75	1.91	1.65	1.79	0.15	0.24	0.11	0.86	< 0.01	0.39	0.09
A:P ratio[3]	2.29	2.49	2.43	2.45	0.09	0.35	0.10	0.08	< 0.01	0.23	0.13

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]High starch: 209 g/kg of starch in DM supplement.

[2]Low starch: 38 g/kg of starch in DM supplement.

[3]Acetate to propionate ratio.

In relation to protozoa numbers, there were no interactions between starch level and oil supplementation on population of Entodinium (P = 0.75), Dasytricha (P = 0.94), Isotricha (P = 0.69), Polyplastron (P = 0.22), Diploplastron (P = 0.59), and total protozoa (P = 0.80). Animals fed with high-starch supplements showed greater Entodinium population (3.79%, $P < 0.01$) and total protozoa (3.26%, $P < 0.01$) than those supplemented with low-starch. Furthermore, the addition of oil in the supplements, independently of starch level utilized, decreased the population of Dasytricha ($P < 0.01$), Polyplastron ($P < 0.21$), and Diploplastron (P = 0.04; Table 5).

Table 5. Effect of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on rumen fluid protozoa numbers of Nellore steers on pasture during finishing phase

Protozoa (n x 10^4 ml^{-1})[3]	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Entodinium	5.75	5.83	5.55	5.60	0.07	< 0.01	0.28	0.75
Dasytricha	4.13	4.48	4.32	4.66	0.11	0.10	< 0.01	0.94
Isotricha	3.41	3.64	3.53	3.64	0.15	0.66	0.24	0.69
Polyplastron	3.63	3.89	3.48	4.01	0.14	0.88	< 0.01	0.22
Diploplastron	3.64	3.95	3.60	4.12	0.19	0.71	0.04	0.59
Total protozoa	5.77	5.88	5.59	5.67	0.06	< 0.01	0.11	0.80

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[1]High starch: 209 g/kg of starch in DM supplement.
[2]Low starch: 38 g/kg of starch in DM supplement.
[3]Log^{10} of number of protozoa.

A significant interaction between starch level and oil supplementation was observed for *Ruminococcus albus* (P = 0.0120). Supplementing the animals with low-starch without oil increased the numbers of *Ruminococcus albus* compared with the other supplements. In addition, there was also interaction between starch × oil for *Selenomonas ruminantium* (P = 0.0003), once, low-starch supplement, with or without oil, decreased the number of *Selenomonas ruminantium* of Nellore steers. Moreover, the addition of oil in the supplements decreased the number of *Fibrobacter succinogenes* (P < 0.0001), *Ruminococcus flavefasciens* (P < 0.0001), and *Archeas* (P < 0.0001), but increased of *Anaerovibrio lipolytica* (P < 0.0001), independently of starch level used. On the other hand, animals supplemented with high-starch showed lower population of *Archeas* than those supplemented with low-starch (P < 0.0001; Table 6).

Table 6. Effect of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on relative proportion (%) of cellulolytic bacteria and methanogenic arqueas of Nellore steers on pasture during finishing phase

Item	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Fibrobacter succinogenes	0.0776	0.1066	0.0696	0.1028	0.0034	0.1085	< 0.0001	0.5221
Ruminococcus albus	0.0032[c]	0.0093[b]	0.0033[c]	0.0100[a]	0.0001	0.0021	< 0.0001	0.0120
Ruminococcus flavefaciens	0.0030	0.0208	0.0035	0.0211	0.0003	0.2119	< 0.0001	0.7463
Anaerovibrio lipolytica	0.0194	0.0021	0.0207	0.0018	0.0004	0.2422	< 0.0001	0.1113
Selenomonas ruminantium	0.0693[b]	0.0788[a]	0.0302[c]	0.0300[c]	0.0007	< 0.0001	0.0003	0.0003
Archeas	0.7139	0.8759	0.8178	1.0043	0.0065	< 0.0001	< 0.0001	0.1239

[a–c]Means within a row with different superscripts differ (P < 0.05).
[1]High starch: 209 g/kg of starch in DM supplement.
[2]Low starch: 38 g/kg of starch in DM supplement.

4. DISCUSSION

This study evaluated the effect of starch-based supplementation level combined with oil on intake, digestibility, rumen fermentation parameters, and rumen microbiota of Nellore steers grazing *Brachiaria brizantha cv.* Xaraés during the finishing phase. Animals supplemented with low-starch supplement combined without oil increased digestibility of CP, and numbers of *Ruminococcus albus*, but decreased the number of *Selenomonas ruminantium*. Moreover, independent of starch level used, the addition of oil in the diet was associated with decreased intake and digestibility of nutrient, acetate concentration, protozoa population, and fibrolytic bacterial population of rumen.

Negative effect of dietary oil on intake is mainly due to a depressive effect on cellulolytic bacteria, ruminal digestion or to a low palatability of oil supplements (Doreau and Chilliard, 1996). It was the case in the present study, since the animals supplemented with oil reduced the intake of DM (5.59%), OM (5.48%), and NDF (8.52%) independently of starch level used. In addition, there was a reduction in the digestibility of DM (6.01%) and NDF (5.12%) in relation other animals fed without oil. This reduce in the intake when oil was fed may be due to a decline in fibrolytic bacteria (Nagaraja et al., 1997; Patra and Yu, 2013), rumen protozoal numbers (Sutton et al., 1983; Machmüller et al., 2003), which possibly to leady an increase rumen retention time due to reduced fiber digestion and particle outflow rates (Demeyer, 1987).

Additionally, other factor that can affect nutrient digestibility with lipid supplementation is the type of basal diet (type and quantity of lipids), thus for grazing animals, forage content, and association between forage × supplement is an important item to be considered (Nörnberg et al., 2004). In this sense, the effect of oil addition in the diet (2.48% to 6.34% EE) was confirmed with the reduction of intake forage DM (8.46%) of animals grazing tropical pasture. The dietary addition of oil at greater than 2 to 3% of DM may decrease digestibility of fibrous feedstuffs such as SH and forage DM, by inhibiting fibrolytic bacteria (Palmquist, 1988). Shain et al. (1993) reported that the rate of digestion of NDF was lower for a combination of SH × oil than for SH alone when fed in a forage-based diet. However, energy provided by oil may compensate for the potential reduction in energy derived from fermentation of SH fiber. Consequently, the intake of digestible energy may be unaffected, or even improved, with higher amounts of dietary oil (Ludden et al., 1995).

On the other hand, animals supplemented with low-starch showed greater (9.80%) intake of NDF than those supplemented with high-starch. This may be explained by the greater NDF digestibility (6.36%) for low-starch diets than high-starch diets. Soybean hulls are high in NDF and ADF, but they are low in lignin (2 % lignin; NRC, 1996), resulting in an *in vitro* DM digestibility that may exceed 90% (Ludden et al., 1995). In addition, SH has a small feed particle size and high

specific gravity (Mertens, 1997) resulting in a more rapid ruminal scape and in a reduction of the ruminal fill (Iraira et al., 2013; Nakamura and Owen, 1989).

Moreover, when starch-based supplementation was combined with oil, there was an alteration in the metabolism of protein in the rumen. The interaction observed between low-starch supplementation and without oil on CP digestibility might be attributed to profile of degradation of soybean hulls (e.g. pectin; Ludden et al., 1995), which results in a better synchronism between protein and energy, increasing efficiencies of microbial protein synthesis in the rumen of animals on grazing pasture (Sinclair et al., 1993; Russel et al., 1992).

In relation to fermentability of diet, it is dependent on the extent and rate of OM digestibility. Therefore, the use of feedstuffs high in sugars, starch, and potentially digestible NDF (e.g. pectin) in the diet of ruminant can increase the concentration of fermentable OM (Allen, 1997). Highly fermentable feeds results in a rapid production of VFA that reduce ruminal pH when the rate of production exceeds buffering capacity and absorption (Russell and Wilson, 1996; Aschenbach et al., 2011). However, the current results showed that oil supplementation reduced OM digestibility, but there was no main effects or interactions between starch × oil on rumen pH. This may be due the starch-based supplements in our experiment have increased the number of Entodinium and total protozoa, once the presence of a high population of protozoa, especially Entodioniomorphs, may contribute to stabilizing ruminal pH and slow ruminal fermentation of carbohydrates (Kurihara et al., 1978). Because protozoa readily engulf ruminal bacteria and starch granules, they may reduce the formation of bacterial slime in the rumen and retard acid production (Bonhomme, 1990; Dehority, 1998).

As concerns to protozoa population, animals fed oil supplements decreased the number of Dasytricha (7.55%), Polyplastron (10.00%), and Diploplastron (10.28%) in the rumen in relation other treatments. Several studies showed that dietary lipids reduce protozoa concentrations in the rumen (Firkins et al., 2007; Machmüller and Kreuzer, 1999; Newbold and Chamberlain, 1988). The toxicity of high dietary oil concentrations to rumen protozoa is due to their limited ability to absorb and transform lipids, resulting in swelling and consequent rupture of the protozoa cells (Girard and Hawke, 1978; Williams, 1989).

Regarding ruminal ammonia was not affected by interaction between starch-based supplementation level and oil in the present experiment. Brokaw et al. (2001) suggested that increase ruminal NH_3-N for cattle receiving oil supplementation was attributed to decrease demand for NH_3-N uptake to support microbial protein synthesis. Therefore, how the concentration of NH_3-N in the rumen results from the balance between production, absorption and incorporation of amino N into microbial protein (Russell et al., 1983), not have been changed by interaction between starch-based and oil supplementation in the current experiment.

In our study, ruminal NH_3-N concentrations were range between 19.46 to 20.71 mg/dL. These concentrations were higher to 15 mg/dL suggested by Detmann et al. (2009) to maximize productive efficiency for cattle fed low-quality tropical forage, but Leng (1990), who suggested concentrations of 20 mg/dL to optimize voluntary intake under tropical conditions, reported a similar pattern. In this sense, our data suggest that there was not a deficiency of NH_3-N in ruminal fluid for maximizing voluntary intake, productive efficiency, and microbial protein synthesis.

Volatile fatty acids concentrations was not affected by interaction between starch level and oil supplementation. However, oil supplementation reduced acetate concentration (9.25%) in the rumen fluid of animals. Probably this may indicate inhibitory activity of oil on ruminal cellulolytic bacteria, allowing an incomplete digestion of NDF (Doreau and Ferlay, 1995). Moreover, concentrations of total VFA, acetate, propionate, butyrate, and valerate increased immediately after feeding, which would be typically expected in animals supplemented on grazing pasture, because manipulation of the diet by increasing the proportion of concentrate is a strategy to optimize ruminal fermentation by increasing the amount of OM digestible, and consequently more substrate for rumen microbial population (Doreau et al., 2011).

Additionally, within this study, we note an increase of *Ruminococcus albus* population within the rumen of steers fed with low starch without oil, which may be due the growth or the activity of fibrolytic bacteria that have been positively favored by this diet, because the low-starch diets had more fibrous material than the high-starch diets.

High-starch diets lead to a decreased efficiency of fiber digestion, associated with a decreased number of some fibrolytic bacteria, i.e. *Ruminococcus flavefasciens* (Tajima et al., 2001), *Fibrobacter succinogenes* (Tajima et al., 2001; Brown et al., 2006; Fernando et al., 2010) and *Butyrivibrio fibrisolvens* (Fernando et al., 2010), and a shift toward more lactate producers like lactobacilli (Brown et al., 2006). Similarly, enrichment of diets with lipids have also negatively affect the fibrolytic activity of ruminal microbiota (Brooks et al., 1954). Moreover, there was also interaction between starch × oil for *Selenomonas ruminantium*, once, low-starch supplements, with oil or not, decreased the number of *Selenomonas ruminantium* of Nellore steers. This may be due the fact that *Selenomonas* utilize starch for growth (Tajima et al., 2001), consequently if it has more substrate available, the diets with high-starch increases the number of these bacteria in relation to the other diets.

5. CONCLUSION

High- or low-starch supplements with or without oil have a similar feeding value when used to animals grazing pasture, as indicated by no differences on intake, digestibility, rumen fermentation parameters, and protozoa population. Soybean grains supplementation decrease intake,

digestibility, acetate production, protozoa population, and fibrolytic rumen bacteria. The use of soybean hulls without oil supplementation may be effective to increase digestibility of CP, and *Ruminococcus albus* of finishing Nellore steers grazing *Brachiaria brizantha* cv. Xaraés during the dry season.

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CHAPTER 7

O artigo a seguir está redigido conforme normas de publicação do *Meat Science* exceto o posicionamento das tabelas.

EFFECT OF STARCH-BASED SUPPLEMENTATION LEVEL COMBINED WITH OIL ON FATTY ACID PROFILE, MEAT QUALITY TRAITS OF YOUNG NELLORE BULLS

ABSTRACT: The fatty acid intake, fatty acid composition, and meat quality traits of 60 young Nellore bulls fed diets with two levels of starch-based supplement with or without a source of oil (ground soybean; GS). The supplements were corn without GS, corn associated with GS, soybean hulls (SH) without GS, and SH associated with GS. There were interaction between starch-based supplementation level and oil to intake of vaccenic ($P < 0.01$), linoleic ($P < 0.01$), Total PUFA ($P = 0.01$). Meat from animals supplemented with-high starch and without oil increased the percentage of vaccenic acid ($P = 0.01$). The use of low-starch supplements with oil increases intake of linoleic and total PUFA. Starch-based or oil supplementation not affect the myristic or palmitic acid content in the *longissimus dorsi* muscle. Oil supplementation increases level of stearic acid and the n-6/n-3 ratio, but decreases percentage of linolenic acid in muscle of Nellore bulls grazing *Brachiaria brizantha* cv. Xaraés during the dry season.

Key words: lipids, meat, ruminant, soybean hulls

1. INTRODUCTION

Improving the nutritional quality of beef is a key challenge for the animal production and beef industry. Despite extensive biohydrogenation (BH) of dietary fatty acid in the rumen, practices such as the nutrient composition of the diet, can change the fatty acid profile of meat, especially by a greater deposition of polyunsaturated fatty acids (PUFA) in muscles to give a greater n-6/n-3 ratio, and make them more attractive for human health reasons (Dewhurst et al., 2003; Wood et al., 2008; Doreau et al., 2011).

Ruminal biohydrogenation of the predominant fatty acid in pasture (C18:3n-3) also leads to production of C18:1trans-11 and ultimately to conjugated linoleic acids (CLA) in tissue (Realini, Duckett, 2004; Descalzo et al., 2005). Supplementation with soybean grains in finishing beef cattle diet is an interesting nutritional management to increase energy density, and production of CLA and vaccenic acid, with the vaccenic acid potentially being additional substrate for the endogenous synthesis of c9, t11 CLA (Harfoot et al., 1973; Scollan et al., 2014; Burnett, et al., 2012).

On the other hand, when beef cattle are fed high a level of grain with highly fermentable starch, there is a shift in rumen microflora, a reduction in rumen pH and a shift in the BH pathways towards producing t10-18:1 instead of vaccenic acid (Bauman and Griinari 2003), since the final step in the BH is influenced by pH (Troegeler-Meynadier et al. 2006).

Several trials have studied the effect of concentrate type on performance in fattening beef cattle (Brennan et al., 1987; Bartoň et al., 2007; Costa et al., 2013), but very few analyzed this effect while dissociating the impact of energy intake from the type of energy source

(Mueller et al., 2011). Thus, type of concentrate energy may modify dietary digestive and metabolic efficiency as well as absorbed nutrient profile; consequently, significant differences in muscle metabolism, and carcass composition (Hocquette et al., 2007).

However, there are no studies to date that evaluate the effects of starch-based supplementation level combined with oil on fatty acids profile and quality traits of meat from young Nellore bulls grazing tropical pasture.

The hypothesis of the present study is that when combined with oil, corn starch is fermented more rapidly in the rumen than starch from soybean hulls, lower ruminal pH and rates of ruminal BH, increasing supply of unsaturated fatty acids in the gut, and consequently improve meat fatty acid profile. This study evaluated the effects of two levels of starch-based supplement combined with or without oil on fatty acid intake, fatty acid composition, and meat quality traits of young Nellore bulls grazing *Brachiaria brizantha cv.* Xaraés during the finishing phase.

2. MATERIALS AND METHODS

The protocol used in this experiment was in accordance with the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal) of the Faculty of Agriculture and Veterinary Sciences – São Paulo State University (UNESP) – Jaboticabal campus (protocol number 021119/11).

2.1. Animals, diet and management

The experiment was conducted at the University Estadual Paulista (UNESP, Jaboticabal, SP, Brazil) from December 2012 to October 2013 and was divided into two phases: the 1st growth phase was characterized by the rainy season (December 2012 to May 2013) and used 60 Nellore bulls, with an average age of 15 months and an initial body weight (IBW) of 284 ± 38 kg. The 2nd finishing phase was characterized by the dry season (May to October 2013) where we used the same animals from the first phase (n = 60, BW = 424 ± 34 kg).

Initially, the animals were weighed, identified and treated against ecto- and endoparasites by administration of ivermectin 1% (Ivomec®, Merial, Paulínia, BR), and were allocated into 12 paddocks, with 1.8 ha, consisting of *Brachiaria brizantha cv*. Xaraés. The animals were distributed in a completely randomized design (five animals per paddock and three paddocks per treatment) with three replicates per treatment.

The diets used consisted of two levels of starch-based supplement with or without a source of oil (ground soybean; GS). The supplements were corn without GS, corn associated with GS, soybean hulls (SH) without GS, and SH associated with GS. The proportion of ingredients and

chemical composition of supplements of 1st phase are presented in Appendix 1; and in the Table 1 are presented the proportion of ingredients, chemical composition, and fatty acid profile supplements of 2nd phase.

Table 1. The ingredient proportions, chemical composition, and principal fatty acids of supplements and pasture during finishing phase (% DM basis)

Item		High Starch		Low Starch		Pasture[1]
		Oil	No Oil	Oil	No Oil	
Ingredient proportions						
Ground corn*		18.5	31.0	0.00	0.00	-
Soybean meal		0.00	38.5	0.00	37.0	-
Soybean hulls		0.00	0.00	18.5	32.5	-
Ground soybean*		51.0	0.00	51.0	0.00	-
Crude glycerin		28.0	28.0	28.0	28.0	-
Commercial premix[2]		2.50	2.50	2.50	2.50	-
Chemical composition						
Dry matter		90.2	89.3	90.3	89.4	-
Organic matter		92.3	92.2	91.7	91.3	92.6
Crude protein		22.9	22.3	23.9	23.6	9.01
NDF		12.7	11.1	21.9	27.1	65.8
Starch[3]		17.20	24.69	4.45	3.29	-
Ether extract		12.4	3.62	11.8	2.58	2.12
Gross energy, *Mcal/kg DM*		5.08	4.62	4.98	4.45	4.02
Fatty acids	*Lipid number*					
Palmitic	C16:0	15.43	20.30	14.36	17.53	29.75
Stearic	C18:0	4.46	4.89	4.01	4.08	5.01
Oleic	C18:1 cis-9	33.69	27.20	27.87	22.83	8.23
Vaccenic	C18:1 cis-11	1.73	1.25	1.55	2.44	----
Linoleic	C18:2	42.51	41.45	47.83	48.62	10.80
Linolenic	C18:3	2.18	2.35	3.33	4.07	13.30
SFA		19.89	26.83	18.80	22.03	42.77
UFA		80.11	72.98	80.58	77.97	34.85
MUFA		35.42	28.99	29.42	25.27	9.29
PUFA		44.69	43.99	51.16	52.69	25.56

[1]Average and standard deviation of the mean of samples obtained by technique of simulated grazing in five periods.
[2]120 g calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1,220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, and 300 mg fluorine.
[3]Calculated based on ingredient values from Valadares Filho et al., 2010.
*Ground in a hammermill fitted with screen size of 3.0 mm (fine).

Crude glycerin is a byproduct from the biodiesel agroindustry and can be used in ruminant diets without compromising intake and performance (Parsons et al., 2009; Drouillard, 2012). This byproduct was used in all supplements to replace (28% of DM) corn or SH. Crude glycerin (83.90%

glycerol, 1.75% ether extract, 4.30% ash, and 12.01% water) was acquired from a soybean-oil-based biodiesel production company (Cargill, Três Lagoas, Mato Grosso do Sul, Brazil).

Animals were supplemented in collective feeders, at the rate of 500 g/100 kg BW in the 1st phase and 1000 g/100 kg BW in the 2nd phase, daily, at 1000 h and had ad libitum access to water and shade. Every 28 days, the animals were weighed after a 16-h period of withdrawal from feed and water, and this BW was used to adjust the amount of supplement.

Grazing method used was continuous stocking with variable stocking rate ("put and take" stocking), maintaining a sward height of 35 cm. Forage height was randomly measured weekly by 80 points using a graduated stick in each paddock (Barthram, 1985). The simulation of grazing per paddock was performed every 28 days. Samples to address herbage chemical composition were obtained by hand plucking methodology to simulate the diet consumed by the bulls (Johnson, 1978). Every 28 days in each paddock, the average height of 80 points, using a graduated stick (Barthram, 1985), was utilized for sampling 4 sites, where all forage included within the perimeter of the rising plate (0.25 m^2) was collected by clipping at 5 cm above soil level from sites that represent the mean forage mass of paddock.

2.2. Intake estimation

Intake of thirty-six animals were estimated using the marker method: lignin isolated, purified, and enriched from *Eucalyptus grandis* (LIPE®) and indigestible neutral detergent fiber (iNDF) were used to estimate the excretion of fecal matter (as dry weight), and forage intake, respectively. The intake of concentrate was obtained through the individual supply of supplement, calculated according to the body weight of the animal. Lignin isolated, purified, and enriched from *Eucalyptus grandis* was provided for 7 days by oral administration of a 500 mg bolus, with 4 days to stabilize fecal excretion of the marker, and in the last 3 days for sample collection (Saliba, 2005).

Fecal samples were collected during 3 days, directly from the rectum, at 1600, 1100 and 0700 h on the first, second and third day of collection, respectively. The fecal samples were dried at 55°C for 72-h and ground in a Wiley mill (Thomas Scientific) to pass through a 1-mm screen and composited proportionately on each of 3 d of sampling, within each animal, based on fecal dry weights. Approximately 10 g of each composited sample of feces was sent to the Federal University of Minas Gerais (Belo Horizonte, MG, Brazil) to estimate the total daily fecal output by 2 methods of LIPE measurement as described by Saliba (2005). Individual concentrate intake was estimated by dividing the total concentrate provided by the number of animals in each paddock.

The individual intake of forage were estimated using the internal marker iNDF. The samples of feces, forage, and concentrate were placed in Ankom bags (Filter bag F57; Ankom Technology, Fairport, NY, USA) and incubated in the rumen of a fistulated Nellore animal for a period of 288-h (Valente et al., 2011). When the bags were withdrawn from the rumen, they were soaked in water for 30 min and gently washed by hand under running water until the wash water ran clear. The bags were then placed in an Ankom^{200} fiber Analyzer (Ankom Technology, Fairport, NY, USA), according to the methods described by Van Soest et al. (1991), and the iNDF was determined by weighing the bags with a digital scale after drying them in an oven, first at 55°C for 72-h and then at 105°C for 12-h. The residue was considered the iNDF. Individual forage intakes were estimated by subtracting marker excretion from the concentrate from the total iNDF excretion and dividing that difference by the concentration of the marker in the forage.

2.3. Slaughter and sample collection

After 270 days of feeding, all the animals were slaughtered at commercial beef plant with 546 ± 43 kg of shrunk body weight. Preharvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997).

Twenty-four hours after the slaughter, samples of *Longissimus dorsi* muscle were collected from the left side of the carcass from the 12th rib, for chemical composition, fatty acid profile, and physical-chemical analysis. All samples were vacuum-packaged and held at -20°C until analysis procedure.

2.4. Fatty acid composition

To determine the fatty acid composition of the fresh meat, samples of the transversal section were collected from the longissimus muscle, freeze-dried, and frozen for lipid extraction and methylation. The fatty material was extracted using a mixture of chloroform–methanol, as reported by Bligh and Dyer (1959) and the fatty acid methyl esters (FAME) were obtained by ISO 5509 method (1978). Qualitative and quantitative measurements of fatty acid content were performed by gas chromatography using a chromatograph (Shimadzu, Kyoto, Japan-Model GC-14B with a Communication Bus Module-CBM 102) with a flame ionization detector (FID) and fused silica capillary column (Omegawax 250), which was 30 m in length and 0.25 mm in diameter and had a film thickness of 0.25 μm (Supelco SP-24136). Helium was used as a carrier gas at a flow of 1 mL/min. A 1-μL aliquot of the sample was injected into a “split” at a division ratio of 1/100 and a

temperature of 250 °C. The temperature of the oven was programmed to remain at 100 °C for 2 min and then increase to 220 °C at 4 °C/min for 25 min, while the detector was at 280 °C. Identification and quantification of the methyl esters of the fatty acids were achieved by comparison with the retention times and concentrations of methyl esters of standard fatty acids.

The activity index were calculated for elongase and Δ^9-desaturase enzymes on fatty acids with 16 and 18 carbons which are responsible for the conversion of SFA with 16 and 18 carbons into their respective correspondents monounsaturated with double bond in carbon 9 as described by Malau-Aduli et al. (1997). The atherogenecity index was calculated as described by Ulbricht and Southgate (1991) as an indicator of risk of cardiovascular disease. Calculations were performed as it follows:

Δ^9 -desaturase activity 16: 100[(C16:1cis9)/(C16:1cis9 + C16:0)];

Δ^9 -desaturase activity 18: 100[(C18:1cis9)/(C18:1cis9 + C18:0)];

Elongase activity: 100[(C18:0 + C18:1cis9)/(C16:0 + C16:1cis9 + C18:0 + C18:1cis9)]; and

Atherogenic index: [C12:0 + 4(C14:0) + C16:0]/ΣSFA + ΣPUFA).

2.5. Proximate analysis

After thawing at room temperature, the *Longissimus dorsi* muscle samples were lyophilized for 36 hours to obtain homogeneous and moisture-free samples, grounded in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen, and the chemical composition was determined according to the Association of Official Analytical Chemists (AOAC, 1990). Crude protein was quantified by the Kjeldahl method, EE was extracted by the Soxhlet method and the ashes were obtained through a muffle furnace at 550 °C.

2.6. Aging times

From each carcass was collected three 2.54 cm-thick steaks of *Longissimus dorsi* muscle corresponding to three aging times (0, 7, and 14 days *post mortem*), to evaluate each meat quality variable at the respective time, totaling nine samples per animal. In this sense, we used different steaks to evaluate a given variable at each aging time. Samples were identified and vacuum packed in polyethylene bags (water vapor permeability <10 g/m^2/24 h at 38°C and oxygen permeability <40 mL/m^2/24 h at 25°C) to determine the pH, color, water holding capacity (WHC), myofibrillar fragmentation index (MFI), Warner–Bratzler shear force (WBSF), malonaldehyde (MDA, mg/kg of meat), thawing loss (TL), cooking loss (CKL), and total loss of meat at three aging times. Samples referring to the day zero were frozen at -20°C for further analysis of meat quality. Samples of the

times 7 and 14 days were aged in cold chamber (no light) between 0 and 2°C. After the color analysis, the samples were stored at -20 °C prior to the CWL and SF analyses.

2.7. Meat color, pH, and water holding capacity

Determination of the L*, a*, and b* color components during blooming was done after removing the filets from the packaging and exposing them to air for 30 min for oxygenation of myoglobin (Tapp III et al., 2011). The color reading was performed on the surface of the steaks using the CIE L*a*b* system, illuminant D65 and at a standard observation angle of 10º. A Minolta CR-400 colorimeter (Konica Minolta, Osaka, Japan) was used, where L* is the index related to luminosity (L*=0 black, 100 white), a* is the index that ranges from green (−) to red (+) and b* is the index from blue (−) to yellow (+) (Houben, Van Dijk, Eikelenboom, and Hoving-Bolink, 2000). Three readings were performed per slice, and the averages were used in the statistical analysis. The colorimeter was calibrated before analyzing the samples against white and black standards.

The values of pH were performed directly in all samples, using a portable pHmeter (SG2 - ELK, Seven Go™, Mettler Toledo International Inc.), with a penetration electrode by introducing it into a cut 2 to 4 cm depth, made in the *Longissimus* muscle.

After evaluating each steak's color, approximately 2 g was collected to determine the water holding capacity (WHC). This value was the difference between the weights of the sample before and after it was subjected to a pressure of 10 kg for 5 min (Hamm, 1986).

2.8. Myofibril fragmentation indices

Myofibril fragmentation indices (MFI) were determined on fresh muscle according to the procedures of Olson et al. (1976) and modified by Culler et al. (1978). Four grams of minced muscle were homogenized for 30 s in 10 vol (v w-1) of a 2°C isolating medium consisting of 100 mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM MgCl, and 1 mM sodium azide. The homogenate was centrifuged at 1000 x g for 15 min. and then the supernatant was decanted. The sediment was then resuspended in 10 vol (v w-1) of isolating medium using a stir rod, centrifuged again at 1000 x g for 15 min. and the supernatant was decanted. The sediment was resuspended in 2.5 vol (v w-1) of isolating medium and passed through a polyethylene strainer (18 mesh) to remove connective tissue and debris. An additional 2.5 vol (v w-1) was used to facilitate passage of myofibrils through the strainer. The protein concentration of the myofibril suspension was determined by the biuret method as described by Gornall et al. (1949). An aliquot of the myofibril suspension was diluted with an isolating medium to reach a protein concentration of 0.5 ± 0.05 mg

mL-1. Protein concentration was determined by the biuret method. The diluted myofibril suspension was stirred and poured into a cuvette; absorbance of this suspension was measured immediately at 540 nm. Absorbance was multiplied by 200 to give a MFI for each sample.

2.9. Warner-Bratzler shear-force measurement and cook loss

Warner-Bratzler shear force (WBSF) steaks were thawed at 4 °C for 24 h and oven-broiled in an electric oven (Layr, Luxo Inox) preheated to 150 °C. Internal steak temperatures were monitored by 20-gauge copper–constantan thermocouples (Omega Engineering, Stamford, CT, USA) placed in the approximate geometric center of each steak and attached to a digital monitor. When internal steak temperature reached 35°C, the steak was turned over and allowed to reach an internal temperature of 70°C before removal from the oven. Cooked WBSF steaks were cooled for 24 h at 4 °C (AMSA (American Meat Science Association), 1995). Five round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibers (AMSA (American Meat Science Association), 1995). Each core was sheared once through the center, perpendicular to the fiber direction by a Warner–Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS - USA). Cook loss was evaluated on the steaks that were also used for WBSF measurement. Total cooking loss was calculated as the difference between the weight of the steaks before and after oven broiling, thawing loss was calculated as the difference between the weight of the steaks before and after thawing, and total loss was calculated by adding up the cooking loss and thawing loss.

2.10. Thiobarbituric acid-reactive substances (TBARS)

The determination of thiobarbituric acid-reactive substances (TBARS) was performed according to Tarladgis et al., 1960. Fifty-gram meat samples were identified and vacuum packed in polyethylene bags for aging time of 0, 7, and 14 days at 1°C in a Bio-Chemical Oxygen Demand (BOD) incubator. A 10-g meat sample was first ground in a multiprocessor, and 0.2 mL of BHT (butylated hydroxytoluene) antioxidant (0.03%) and 50 mL of distilled water and 1 mL of an antifoaming solution were added (Sigma A5758, São Paulo, Brazil). The samples were then ground again and homogenized for 1 min.

After homogenization, the samples were transferred to a 250 mL flask containing porcelain pieces, and 50 mL of a 4 M HCl solution was added. Subsequently, the samples were distilled in a blanket heater at 100°C until 50 mL of the distillate was collected. Five milliliters of the distillate was transferred to a test tube, and 5 mL of 0.02 M TBA solution was then added. The test tubes

were kept in a boiling water bath for 35 min, and the absorbance was measured at 530 nm in a spectrophotometer (Hitachi High Technologies America, Inc., model U-2,900, Pleasanton, USA). The TBARS value, expressed in milligrams of malonaldehyde per kilogram of meat, was obtained using a conversion factor based on a standard curve using 1, 1, 3, 3-tetraethoxypropane (TEP).

2.11. Statistical analysis

The experimental design was completely randomized in a 2 × 2 factorial arrangement (high- or low-starch, with or without a source of oil). Each paddock was considered as the individual experimental unit (3 animals per paddock and 3 paddocks per treatment), and the model effects included treatment.

In relation to aging times, pH, coloration, water holding capacity, myofibrillar fragmentation index, shear force, lipid oxidation, thawing loss, cooking loss, and total loss data were analyzed as repeated measures over time, using the PROC MIXED procedure of SAS. The model included the fixed effects of treatments and days, along with their interaction. Analysis was conducted via repeated measures by days.

Data were analyzed with starch level and oil inclusion as fixed effects and the residual error as a random effect using PROC MIXED of the SAS statistical software (SAS Inst. Inc., Cary, NC). Homogeneity of the data was verified using the UNIVARIATE procedure of SAS. Studentized residuals were plotted against the predicted values using the plot procedure to analyze data for outliers. The LSMEANS statement of the mixed procedure of SAS was used to calculate mean values. When the treatments were significant, the means were compared with Fisher's tests using the PDIFF option in LSMEANS command. The level of significance used to assess differences among means was $\alpha = 0.05$.

3. RESULTS

There were no interactions between starch-based supplementation level and oil with regard to intake of DM ($P = 0.90$), forage DM ($P = 0.95$), supplement DM ($P = 0.87$), and EE ($P = 0.56$). There was effect of starch and oil on intake of EE ($P < 0.01$). The addition of oil increased the intake of EE ($P < 0.01$) independently of starch level used, and animals supplemented with high starch showed greater intake EE ($P < 0.01$) than those supplemented with less starch (Table 2).

Table 2. Effects of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on intake of principal fatty acids (g/d) of young Nellore bulls on pasture during finishing phase

Item	Lipid number	High Starch[2]		Low Starch[3]		SEM	*P-value*		
		Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Intake, kg/d									
Dry Matter		12.70	12.81	12.63	12.65	0.37	0.75	0.86	0.90
Forage DM		7.73	7.70	7.77	7.70	0.39	0.96	0.90	0.95
Supplement DM		4.97	5.11	4.86	4.94	0.14	0.37	0.48	0.87
Ether extract		0.78	0.34	0.74	0.29	0.01	< 0.01	< 0.01	0.56
Fatty acid[1]									
Lauric	12:0	7.72	6.45	7.21	7.91	1.17	0.69	0.81	0.42
Myristic	14:0	3.30	3.62	3.73	4.08	0.81	0.61	0.70	0.98
Pentadecanoic	15:0	6.67	5.37	7.23	7.78	1.21	0.25	0.76	0.46
Palmitic	16:0	166.53	100.66	149.47	90.72	7.04	0.09	< 0.01	0.62
Stearic	18:0	37.28	25.23	33.52	14.62	3.82	0.09	< 0.01	0.39
Palmitoleic	16:1 trans-9	2.85	3.94	2.41	3.03	0.10	0.03	0.02	0.20
Oleic	18:1 cis-9	222.14	74.33	180.05	46.23	5.96	< 0.01	< 0.01	0.27
Vaccenic	18:1 cis-11	10.68^{a}	2.30^{d}	8.89^{b}	3.11^{c}	0.16	0.01	< 0.01	< 0.01
Linoleic	18:2 n -6	281.94^{b}	104.38^{c}	301.25^{a}	86.11^{d}	4.49	0.91	< 0.01	< 0.01
Linolenic	18:3 n-3	36.38	33.85	55.87	35.84	5.52	0.08	0.07	0.15
Total SFA		220.41	144.52	200.56	124.74	9.47	0.06	< 0.01	0.99
Total UFA		558.81	224.27	546.87	176.08	9.29	0.01	< 0.01	0.08
Total MUFA		234.72	81.99	189.75	50.35	6.34	< 0.01	< 0.01	0.32
Total PUFA		324.09^{b}	142.28^{c}	357.12^{a}	125.73^{c}	7.76	0.31	< 0.01	0.01

$^{a\text{-}c}$Means within a row with different superscripts differ by Fisher's test at $\alpha = 0.05$.

[1]SFAs = saturated fatty acids; UFAs = unsaturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.

[2]High starch: 209 g/kg of starch in DM supplement.

[3]Low starch: 38 g/kg of starch in DM supplement.

With regard to intake of fatty acid profile, there were interaction between starch-based supplementation level and oil to intake of vaccenic ($P < 0.01$), linoleic ($P < 0.01$), Total PUFA ($P = 0.01$). However, differences were no observed on intake of lauric ($P = 0.42$), myristic ($P = 0.98$), pentadecanoic ($P = 0.46$), palmitic ($P = 0.62$), stearic ($P = 0.39$), palmitoleic ($P = 0.20$), oleic ($P = 0.27$), linolenic ($P = 0.15$), total SFA ($P = 0.99$), total UFA ($P = 0.08$), and total MUFA ($P = 0.32$) among animals fed starch-based supplementation level and oil (Table 2).

Animals supplemented with low- or high-starch combined with oil had no differences on chemical composition in meat ($P > 0.05$). However, there was main effect of starch supplementation on percentage of protein ($P = 0.04$) and ether extract ($P < 0.01$), once, animals supplemented with high-starch were 4.37 and 41.35 % greater than those supplemented with low-starch, respectively (Table 3).

Table 3. Effects of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on chemical composition (%) of *Longissimus dorsi* muscle from young Nellore bulls on pasture during finishing phase

Item	High Starch[1]		Low Starch[2]		SEM	*P-value*		
	Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
Moisture	70.73	70.61	71.55	72.33	0.59	0.06	0.59	0.47
Ashes	1.35	1.40	1.48	1.41	0.03	0.08	0.74	0.11
Protein	22.99	22.85	22.19	21.66	0.42	0.04	0.45	0.66
Ether Extract	3.28	2.62	1.70	1.77	0.28	< 0.01	0.32	0.23

[1]High starch: 209 g/kg of starch in DM supplement; [2]Low starch: 38 g/kg of starch in DM supplement.

There were no interactions between starch × oil for pH ($P = 0.75$), color yellowness (b*; $P = 0.12$), water holding capacity (WHC; $P = 0.86$), myofibrillar fragmentation index (MFI; $P = 0.21$), warner-bratzler shear force (WBSF; $P = 0.05$), malonaldehyde (MDA; $P = 0.29$), thawing loss (TL; $P = 0.18$), cooking loss (CKL; $P = 0.55$), and total weight loss ($P = 0.18$). However, there was an interaction between levels of starch and oil for color values of lightness (L*; $P = 0.02$), and redness (a*; $P = 0.01$). In addition, for the storage period, there was interaction between aging time and the treatments on color redness, yellowness, water holding capacity, myofibrillar fragmentation index, warner-bratzler shear force, malonaldehyde, thawing loss, and total weight loss ($P < 0.05$; Table 4).

Table 4. Effects of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on pH, color (L*, a* and b*), water holding capacity (WHC), myofibrillar fragmentation index (MFI), Warner–Bratzler shear force (WBSF), malonaldehyde (MDA, mg/kg of meat), thawing loss (TL), cooking loss (CKL), and total loss of meat from young Nellore bulls on pasture

Item	High Starch[1]		Low Starch[2]			Aging time (days)				*P-value*			
	Oil	No Oil	Oil	No Oil	SEM	0	7	14	SEM	Time	Starch	Oil	Starch × Oil
pH	5.91	5.92	6.12	6.08	0.07	5.98	6.04	5.99	0.07	0.55	0.02	0.81	0.75
L*	37.53^{a}	35.22^{ab}	33.67^{b}	36.66^{ab}	1.01	35.03	36.03	36.26	1.08	0.07	0.22	0.71	0.02
a*	13.60^{b}	15.17^{a}	14.22^{b}	14.63^{ab}	0.25	17.41^{a}	13.25^{b}	12.57^{b}	0.41	< 0.01	0.83	< 0.01	0.01
b*	12.67	13.00	11.24	12.86	0.46	13.95^{a}	11.67^{b}	11.71^{b}	0.48	< 0.01	0.07	0.03	0.12
WHC	79.94	80.05	79.39	79.41	0.25	78.97^{b}	80.95^{a}	79.16^{b}	0.23	< 0.01	0.03	0.78	0.86
MFI, %	66.73	66.43	65.69	73.36	3.14	52.41^{c}	69.03^{b}	82.71^{a}	3.16	< 0.01	0.34	0.24	0.21
WBSF, kgf	3.97	4.36	3.61	2.83	0.28	4.21^{a}	3.57^{b}	3.29^{b}	0.22	< 0.01	< 0.01	0.48	0.05
MDA, mg/kg	0.80	0.77	0.73	0.75	0.03	0.60^{b}	0.67^{b}	1.01^{a}	0.05	< 0.01	0.05	0.93	0.29
TL, %	4.55	5.10	4.43	3.76	0.53	2.61^{c}	4.28^{b}	6.48^{a}	0.99	< 0.01	0.12	0.88	0.18
CKL, %	19.96	20.17	19.77	18.55	1.18	19.04	20.03	19.77	1.04	0.60	0.45	0.67	0.55
Total loss, %	22.83	23.36	24.59	21.96	1.14	21.12^{b}	23.42^{ab}	25.04^{a}	1.21	0.02	0.87	0.36	0.18

[a-c] Means within a row with different superscripts differ by Fisher's test at $\alpha = 0.05$.

[1]High starch: 209 g/kg of starch in DM supplement.

[2]Low starch: 38 g/kg of starch in DM supplement.

With regard to saturated fatty acids, there were no interactions between starch level and oil supplementation on myristic ($P = 0.62$), pentadecanoic ($P = 0.07$), palmitic ($P = 0.99$), heptadecanoic ($P = 0.26$), and stearic ($P = 0.38$). Therefore, meat from animals fed oil had greater ($P < 0.01$) level of stearic acid (C18:0) compared to meat from animals fed without oil, independently of starch level utilized (Table 5). In relation to MUFA, no interactions were observed between the treatments ($P > 0.05$). Therefore, meat from animals supplemented with high-starch and without oil had greater percentage of vaccenic acid (C18:1 cis-11; $P = 0.01$) than meat from animals fed with others supplements (Table 5).

Table 5. Effects of supplements containing high- or low-starch sources with or without oil (Oil or No Oil) on principal fatty acids saturated and unsaturated (%) found in the muscle longissimus of young Nellore bulls on pasture during finishing phase

Fatty acid[1]	Lipid number	High Starch[2]		Low Starch[3]		SEM	*P-value*		
		Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
SFA									
Myristic	14:0	2.26	1.94	1.84	1.77	0.24	0.26	0.46	0.62
Pentadecanoic	15:0	0.56	0.51	0.46	0.64	0.05	0.81	0.30	0.07
Palmitic	16:0	22.11	21.29	20.13	19.31	1.29	0.16	0.54	0.99
Heptadecanoic	17:0	1.33	1.17	1.07	1.30	0.16	0.71	0.83	0.26
Stearic	18:0	18.00	14.84	18.19	16.36	0.72	0.27	< 0.01	0.38
MUFA									
Myristoleic	14:1 cis-9	0.50	0.51	0.47	0.57	0.05	0.84	0.35	0.49
Palmitoleic	16:1 trans-9	1.84	2.25	1.65	1.59	0.17	0.03	0.33	0.20
Heptadecanoic	17:1 cis-10	1.04	1.35	0.97	1.39	0.08	0.87	< 0.01	0.55
Heptadecanoic	17:1 trans-10	2.63	2.47	2.84	3.68	0.64	0.30	0.61	0.46
Oleic	18:1 cis-9	30.00	33.25	30.12	26.39	2.43	0.20	0.92	0.18
Vaccenic	18:1 cis-11	1.02[c]	1.38[a]	1.07[c]	1.24[b]	0.03	0.22	< 0.01	0.01
Vaccenic	18:1 trans-11	1.26	1.39	1.36	1.38	0.17	0.80	0.68	0.76

[a-c]Means within a row with different superscripts differ by Fisher's test at $\alpha = 0.05$.
[1]SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids.
[2]High starch: 209 g/kg of starch in DM supplement.
[3]Low starch: 38 g/kg of starch in DM supplement.

As regards to PUFA, there were no interactions between starch × oil for linoleic ($P = 0.83$), CLA ($P = 0.24$), linolenic ($P = 0.70$), eicosatrienoic ($P = 0.65$), arachidonic ($P = 0.95$), EPA ($P = 0.52$), DTA ($P = 0.57$), and DHA ($P = 0.43$). However, meat from animals supplemented with oil had lower percentage of linolenic acid ($P = 0.05$) than animals fed without oil. In relation to total fatty acid, differences were no observed on deposition of saturated fatty acids ($P = 0.12$), monounsaturated fatty acids ($P = 0.29$), polyunsaturated fatty acids ($P = 0.91$), unsaturated fatty acids ($P = 0.16$), unsaturated/saturated fatty acids ratio ($P = 0.16$), and n-6/n-3 ratio ($P = 0.68$) among animals fed starch-based supplementation level combined with oil. Therefore, independently of starch level used, oil supplementation increased the n-6/n-3 ratio ($P < 0.01$; Table 6).

Table 6. Effects of supplements containing high- or low-starch sources with or without oil on principal fatty acids polyunsaturated and index of enzymes involved on fatty acids metabolism, atherogenicity and elongase index in muscle *Longissimus* of Nellore bulls on pasture during finishing phase

Fatty acid[1]	Lipid number	High Starch[2]		Low Starch[3]		SEM	*P-value*		
		Oil	No Oil	Oil	No Oil		Starch	Oil	Starch × Oil
PUFA									
Linoleic	18:2 n -6	10.10	8.40	11.25	8.77	1.73	0.69	0.29	0.83
CLA	18:2 cis-9, trans-11	0.54	0.50	0.45	0.49	0.02	0.16	0.93	0.24
Linolenic	18:3 n-3	0.69	0.83	0.72	0.91	0.06	0.49	0.05	0.70
Eicosatrienoic	20:3 n-6, cis-8	0.57	0.55	0.80	0.60	0.18	0.51	0.59	0.65
Arachidonic	20:4 n-6	2.49	2.37	2.72	2.54	0.49	0.71	0.77	0.95
EPA	20:5 n-3	0.35	0.42	0.30	0.48	0.06	0.91	0.15	0.52
DTA	22:4 n-6	0.24	0.26	0.28	0.36	0.04	0.25	0.38	0.57
DHA	22:6 n-3	0.62	0.98	0.70	0.84	0.11	0.81	0.08	0.43
Total SFA		44.29	39.77	41.67	42.49	1.43	0.97	0.26	0.12
Total MUFA		38.32	43.28	38.79	39.98	1.55	0.42	0.10	0.29
Total PUFA		15.09	13.76	16.44	14.52	2.51	0.70	0.56	0.91
Total UFA		53.41	57.04	55.23	54.50	1.33	0.80	0.33	0.16
UFA/SFA		1.21	1.44	1.34	1.31	0.07	0.93	0.26	0.16
n-6/n-3		14.39	9.99	15.00	9.40	1.31	0.99	< 0.01	0.68
Index of Δ^9 -desaturase C16[4]		7.66[b]	9.56[a]	7.57[b]	7.58[b]	0.38	0.02	0.03	0.03
Index of Δ^9 -desaturase C18[5]		62.27	69.12	62.33	61.24	1.93	0.07	0.17	0.07
Elongase index[6]		66.68	67.12	68.87	67.29	0.73	0.14	0.46	0.20
Atherogenicity index[7]		0.52	0.54	0.47	0.45	0.05	0.18	0.94	0.70

[a-c] Means within a row with different superscripts differ by Fisher's test at $\alpha = 0.05$.

[1]SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; UFAs = unsaturated fatty acids.

[2]High starch: 209 g/kg of starch in DM supplement.

[3]Low starch: 38 g/kg of starch in DM supplement.

[4]Index of Δ^9 -desaturase C16 activity = 100[(16:1 cis-9)/(16:1 cis-9+16:0)].

[5]Index of Δ^9 -desaturase C18 activity = 100[(18:1 cis-9)/(18:1 cis-9+18:0)].

[6]Elongase = 100[(C18:0+C18:1 cis-9)/(C16:0+C16:1 cis-9+C18:0+C18:1 cis-9)].

[7]Atherogenicity index = [C12:0+4(14:0)+C16:0]/(ΣSFA+ΣPUFA).

There were no interactions between starch × oil on index of Δ^9-desaturase C18 (P = 0.07), elongase (P = 0.20), and atherogenicity (P = 0.70) in *Longissimus dorsi* muscle. However, meat from animals supplemented with high-starch and without oil had greater index of Δ^9-desaturase C16 (P = 0.03) than meat from animals fed with others supplements (Table 6).

4. DISCUSSION

Animals fed with low-starch (soybean hulls) supplement consumed the same amount of DM as those fed high-starch (corn) when combined with the source of oil (ground soybean). When SH was fed in high-forage (> 50% forage) beef cattle diets, the nutritive value of SH was estimated to be similar to that of corn (Hibberd et al., 1987; Anderson et al., 1988). Thus, despite of SH has high NDF and ADF, is low in lignin (2% lignin; NRC, 1996), resulting in an *in vitro* DM digestibility that may exceed 90% (Ludden et al., 1995), providing the same DM intake between the treatments. These results are consistent with previous studies (Valk et al., 1990; Spörndly, 1991; Jose Neto et al., 2015), which showed no differences in DM intake between fiber- and starch-based supplements.

The chemical composition of meat is determined by several factors such as moisture, ash, crude protein, and total lipids. Animals supplemented with high-starch had greater protein and deposition of intramuscular fat compared to meat from animals fed with low-starch. Some authors reported crude protein percentage in *Longissimus muscle* varying between 21 and 24% (Aricetti et al., 2008; Macedo et al., 2008; Prado et al., 2008; Maggioni et al., 2009), which was confirmed in our study with average values of 22.42 % of protein in meat. The main factors that influence the chemical composition are sex (Arthaud et al., 1977), aging (Dikeman et al., 2013), breed (De Smet et al., 2004), growth rate (Chambaz et al., 2003) and energy intake (Arthaud et al., 1977), and most of them were similar in this study.

In relation to amount of fat in meat, this component presents greatest variations. In general, the quantity of fat deposited is a result of the balance between energy intake and energy expenditure by the animal. If energy intake is greater than the metabolic demands of the animal, this excess will be stored as fat (Johnson et al., 2003). Total lipids in meat of cattle can vary from 2 to 5% (Padre et al., 2007; Kazama et al., 2008). The main end product of starch fermentation is propionic short chain fatty acid. Thus, because propionate is gluconeogenic, the greater production of this acid could increase deposition of intramuscular fat, which uses a high proportion of glucose for the fatty acid synthesis (Gilbert et al., 2003).

Animals fed with high- or low-starch exhibited the similar pH in meat compared to animals supplemented with or without oil, demonstrating the lack of main effect or interaction between treatments. However, high-starch supplement provided low pH in meat that may be due by increasing muscle glycogen, because of its greater post-ruminal absorption as glucose (Armstrong & Beever, 1969; Lindsay, 1981). The pH is an important characteristic to be evaluated as it is responsible for changes in meat quality traits such as color (Wulf et al., 1997). Meat with pH above 6.0, after 24 h *post mortem*, can be dark, firm and have a dry cut surface, characteristics known as DFD (Dark, Firm and Dry). This DFD meat is of inferior quality as the less pronounced taste and the dark colour are less acceptable to the consumer and it has a shorter shelf life, due to the abnormally high pH value, which is conducive to bacterial growth (Priolo et al., 2001).

In general, interaction between starch × oil supplements during finishing period did not influence the beef quality of Nellore bulls grazing pasture. However, muscle from animals supplemented with low-starch and oil showed lower value of color (L*) than other animals. This may be associated with the greater pH value, once that high pH meat has lower L* (lightness), a* (redness), b* (yellowness) values than normal pH meat, indicating that high pH meat is darker and less brown than is normal pH meat (Zhang et al., 2005). Furthermore, at high pH values, muscle enzymes that utilize oxygen become more active, resulting in less oxidation of surface myoglobin and a higher a* (Ledward et al., 1992).

The muscular rate of lipid oxidation may also act as an indicator of the degree of meat pigment susceptibility to oxidation, since there is a close relationship between these two processes (Trout, 2003; McKenna et al., 2005). However, animals receiving high- or low-starch supplements with or without oil did no influence on oxidation of lipids in muscle. A greater oxidation possibly occurred due to the greater PUFA concentrations in the LD muscle from these animals, but our data did not find alterations to PUFA concentrations in muscle. Wood et al. (2004) have reported that increased muscle linoleic and α-linolenic acid concentrations resulted in significant reductions in lipid stability levels after 10 days of storage. This data supports our finding that muscle from animals after 14 days reduced the lipid stability.

The water-holding capacity is defined as the ability of meat to retain its water during application of external forces, such as cutting, heating, grinding or pressing (Lawrie & Ledward, 2006), and greater water-holding capacity in high pH meat than in normal pH meat (Zhang et al., 2005). The lack of effects of interaction between starch-based and oil supplementation on WCH may have occurred as a consequence of the lack of differences on pH

values. The most of water in muscle is maintained by capillary forces between the thick and thin filaments, and WHC of meat is greatly affected by pH (Offer and Trinick, 1983; Offer and Knight, 1988).

Another interesting aspect that contributed to improve the beef quality are the changes in the fatty acid profile, once that a lower proportion of SFA may reduce the incidence of cardiovascular disease in consumers (Breidenstein, 1985; Ladeira et al., 2014). Starch-based or oil supplementation did not affect the myristic or palmitic acid content in the LD muscle. These results have important implications for human health, because myristic and palmitic acids interfere with the normal function of LDL receptors in the liver, reducing LDL removal and increasing its concentration in plasma (Woollett, Spady, & Dietschy, 1992; Wood et al., 2003).

The increases in intake of linoleic and total PUFA by animals fed with low-starch supplement when combined with oil was not sufficient to promote changes in the meat PUFA. A possible explanation for this contradiction may be due to a long rumen transit time for high forage diets, since the forage intake was about 60% of total DM ingested, animals grazing pasture, promoting greater biohydrogenation of PUFA in the rumen (Wood et al., 2008). Linoleic acid is derived entirely from the diet. In ruminants, the fatty acid, which is at high levels in concentrate feedstuffs (e.g. soybean grains) is degraded into monounsaturated and saturated fatty acids in the rumen by microbial biohydrogenation and only a small proportion, around 10% of dietary 18:2 n-6, is available for incorporation into tissue lipids (Wood et al., 2008).

In our study, the supply of oil supplementation or the interaction between starch and oil did not increase the CLA (C18:2 cis-9, trans-11) content in the LD muscle of animals. Our results no corroborate a previous study showing that CLA content was greater (15%) in the LD muscle of animals fed ground soybean (greater levels of linoleic acid, linolenic acid, and total PUFA), which can be explained by a greater biohydrogenation of linoleic acid as a consequence of grinding the oilseed (Ladeira et al., 2014). Generally, during biohydrogenation in the rumen, linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids are converted to CLA, and CLA is converted to vaccenic acid (C18:1 trans-11), which originates stearic acid (C18:0) as the end product (Bauman et al., 2000).

The fatty acids from ω-3 and ω-6 families are essential for humans, as it has not an endogenous synthesis. Thus, the ω-6:ω-3 ratio is recommended to be lower than 4:1 (Wood et al., 2003), and higher values may indicate a non-healthy diet that may lead to a coronary artery diseases (Department of Health of London, 1994). The ω-6:ω-3 ratio observed in muscle of animals fed supplements with oil was 34.03% greater than the without oil, above the recommended

value. These values may be explained by a greater concentration of linoleic acid in muscle from animals fed with oil supplement.

5. CONCLUSION

The use of low-starch supplements with oil increases intake of linoleic and total PUFA. Starch-based or oil supplementation not affect the myristic or palmitic acid content in the *longissimus dorsi* muscle. Oil supplementation increases level of stearic acid and the n-6/n-3 ratio, but decreases percentage of linolenic acid in muscle of Nellore bulls grazing Brachiaria brizantha cv. Xaraés during the dry season.

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APÊNDICE

Apêndice A - IMPLICAÇÕES DO USO DE ALTO OU BAIXO AMIDO, ASSOCIADOS OU NÃO COM O GRÃO DE SOJA MOÍDO EM SUPLEMENTOS PARA BOVINOS DE CORTE EM PASTAGENS DE *BRACHIARIA BRIZANTHA* CV. XARAÉS

O projeto FAPESP Nº 2012/08284-5, foi o que proporcionou a realização dessa tese, intitulada: "**Uso de fontes de energia na suplementação de bovinos de corte em pastagens de *Brachiaria brizantha* cv. Xaraés**". Este projeto está inserido em um amplo projeto temático FAPESP Nº 2011/00060-8, intitulado: "**Balanço de gases de efeito estufa e estratégias de mitigação em pastos de *Brachiaria* submetidos a diferentes manejos**", que por sua vez, teve como principais objetivos estudar o balanço dos gases de efeito estufa no sistema solo, planta e animal na atividade pecuária e o uso de diferentes estratégias de manejo do pasto e suplementação animal como forma de mitigação de gases de efeito estufa e melhoria da qualidade ambiental.

Dentre as alternativas de ingredientes para utilização na suplementação animal, a glicerina bruta têm se destacado como um ingrediente energético com potencial para substituir fontes energéticas, como o milho e a casca de soja, melhorando a rentabilidade da indústria energética (Biodiesel) e minimizando os passivos ambientais.

Nesse sentido, antes da condução desse experimento, foi realizado um projeto FAPESP Nº 2011/06409-2, também inserido no temático supracitado, para avaliar níveis crescentes de inclusão de glicerina bruta (0, 7, 14, 21 e 28 % da MS) na suplementação de bovinos de corte em pastejo de *Brachiaria*: "**Glicerina bruta no suplemento de bovinos de corte criados a pasto**", sendo que, o melhor resultado obtido foi para o nível de 28% de substituição do milho do suplemento, com base na MS.

Diante desse achado, nosso estudo utilizou esse nível de 28% da MS em todos os tratamentos avaliados, independente dos níveis de amido, associados ou não com o grão de soja moído. Nossos resultados comprovaram o potencial da glicerina bruta como ingrediente alternativo, uma vez que, o desempenho predito pelas tabelas de exigências nutricionais (BR-Corte, 2010) foi semelhante aos dados reais encontrados no presente estudo. Assim, obtemos uma importante implicação na cadeia produtiva de alimentos, uma vez que, a glicerina bruta pode ser incorporado na dieta de bovinos de corte à pasto, reduzindo o consumo de milho pelos animais e, consequentemente, disponibilizando uma maior quantidade de milho para se utilizar na alimentação humana. Outro ponto relevante é para a indústria do Biodiesel, pois cria-se um destino adequado para a utilização desse subproduto, não sendo necessário ser descartado no meio ambiente, além de que, pode ser considerado como um adicional para o faturamento das indústrias.

Algumas limitações em relação ao uso desse subproduto líquido:

1- Processo de mistura: a fabricação do suplemento e inclusão da glicerina bruta foi realizada com a utilização de uma betoneira: máquina compacta provida de um tambor giratório, movida à energia elétrica, que prepara o concreto ou mistura as argamassas, geralmente para a construção civil.

2- Fornecimento: logo após finalizar a mistura, os concentrados se encontravam farelados e adequados para serem colocados nos sacos e armazenados. Porém, após dois ou três dias de armazenagem, os concentrados que continham glicerina bruta formavam blocos no formato do saco, o que dificultava a sua remoção para fornecimento para os animais. Portanto, era preciso desintegrar o bloco de concentrado formado dentro de cada saco, que nem sempre voltava novamente ao seu estado totalmente farelado. Diante dessa dificuldade, a estratégia foi misturar os concentrados todos os dias, pouco tempo antes do fornecimento para os animais.

3- Interação com outros ingredientes da dieta: essa interação e aglomeração da glicerina com os outros ingredientes avaliados, se deu de forma mais intensiva quando se utilizou o grão de soja moído. Provavelmente, em função da maior quantidade de óleo nesses concentrados, favoreceram a aglomeração dos suplementos, sendo inviável o fornecimento após longo período de armazenamento.

4- O transporte e armazenamento são feitos em "bombonas" plásticas (250 L) ou pequenos tanques (1.000 L) contendo uma torneira própria. Porém a descarga desses recipientes necessita de máquina, devido ao seu elevado peso.

Figura 1: Recipientes utilizados para armazenar a glicerina bruta; Betoneira – máquina utilizada para misturar os concentrados; Aglomeração da glicerina com os outros ingredientes após dias de armazenamento.

Em relação à associação de níveis de amido com grão de soja moído, embora seja reconhecido que a composição da dieta afeta a contribuição dos ruminantes para a produção de gás de efeito estufa, o Painel Intergovernamental de Mudanças Climáticas, responsável pelo desenvolvimento de metodologias para estimar inventários de emissão global, apenas faz diferenciação entre duas dietas (IPCC, 2006):

1- Dietas com mais 90% de concentrado: taxa de conversão de CH_4 de 3% da energia bruta ingerida e;
2- Dietas com menos de 90% de concentrado: taxa de conversão de CH_4 de 6,5% da energia bruta ingerida.

Esse intervalo pode não representar as condições observadas nos sistemas de produção de ruminantes no Brasil, onde dificilmente são observados níveis de inclusão de mais de 90% de concentrado na dieta e, talvez a amplitude de 0 a 90% de concentrado seja pouco específica para a maior parte do manejo adotado para o rebanho de ruminantes no país, onde a pecuária ainda está baseada em um sistema de produção extensivo, onde os animais permanecem a maior parte das suas vidas em pastagem. Diante disso, esse estudo trabalhou com duas diferentes relações de volumoso:concentrado, representando o sistema brasileiro de produção: período de recria dos animais – estação chuvosa (80:20) e no período de terminação dos animais – estação da seca (60:40).

Para a relação volumoso: concentrado (80:20), os resultados encontrados foram que o valor médio calculado para as emissões de metano por animal foi de 43,83 kg de CH_4/ano e que a energia bruta média perdida na forma de emissões de metano foi de 3,65%. Já para a relação volumoso:concentrado (60:40), a quantidade emitida por animal foi de 47,03 kg de CH_4/ano e que a perda de energia na forma de emissões de metano foi 2,89%. Estes valores estão abaixo dos valores estimados pelo IPCC (2006), o qual afirma que o valor de produção anual para bovinos é de 56 kg de CH_4. Portanto, nossos resultados podem contribuir para novas estimativas do IPCC, para inventários de emissões globais em regiões de pastagens tropicais, com baixa inclusão de concentrado na dieta dos animais.

Apêndice B – TABELA DOS SUPLEMENTOS EXPERIMENTAIS E DA COMPOSIÇÃO QUÍMICA DOS SUPLEMENTOS E DO PASTO

Table 1 - Experimental supplement and chemical composition of supplements and pasture of growing phase (% DM basis)

Item	High Starch		Low Starch		Pasture[1]
	Oil	No Oil	Oil	No Oil	
Ingredient proportions					
Ground corn*	8.90	18.5	0.00	0.00	-
Soybean meal	0.00	49.0	0.00	49.0	-
Soybean hulls	0.00	0.00	8.50	18.5	-
Ground soybean*	58.6	0.00	59.0	0.00	-
Crude glycerin	28.0	28.0	28.0	28.0	-
Commercial premix[2]	4.50	4.50	4.50	4.50	-
Chemical composition					
Dry matter	90.9	88.1	90.2	88.2	89.8
Organic matter	91.7	89.5	90.9	89.2	92.7
Crude protein	27.6	26.5	26.2	26.0	15.9
NDF	13.2	11.0	17.5	20.2	61.2
Starch[3]	11.0	16.3	4.79	3.52	-
Ether extract	13.8	3.18	13.4	2.57	1.31
Gross energy, *Mcal/kg DM*	5.16	4.51	5.07	4.41	4.49

[1]Average and standard deviation of the mean of samples obtained by technique of simulated grazing in five periods.

[2]120 g Calcium, 30 g phosphorus, 25 g sulfur, 80 g sodium, 330 mg copper, 950 mg manganese, 1,220 mg zinc, 24 mg iodine, 20 mg cobalt, 6 mg selenium, and 300 mg fluorine.

[3]Calculated based on ingredient values from Valadares Filho et al., 2010.

[*]Ground in a hammermill fitted with screen size of 3.0 mm (fine).