TAINÁ SILVESTRE MOREIRA

Energy requirements, energetic partition and methane emission from growing Holstein, Gyr and F1 Holstein-Gyr dairy heifers

Pirassununga

2016

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

Departamento:

Nutrição e Produção Animal

Área de concentração:

Nutrição e Produção Animal

Orientador:

Prof. Dr. Paulo Henrique Mazza Rodrigues

De acordo:__________________

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UNIVERSIDADE DE SÃO PAULO

FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Exigências nutricionais, partição de energia e emissão de metano entérico por novilhas leiteiras Holandês, Gir e F1 Holandês-Gir", registrada com o nº 3046/2013, sob a responsabilidade de Paulo Henrique Mazza Rodrigues e Tainá Silvestre Moreira - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto humanos), para fins de pesquisa científica - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ), em reunião de 6/4/2016.

Finalidade	Pesquisa Científica
Vigência da autorização	1º/12/2012 a 1º/12/2013
Espécie/linhagem/raça	Bovinos/Holandês, Gir e F1 Holandês-Gir
Nº de animais	36
Peso/Idade	100 kg /9 meses
Sexo	Fêmeas
Origem	EMBRAPA Gado de Leite

São Paulo, 7 de abril de 2016.

Denise Fantoni

Denise Tabacchi Fantoni
Presidente

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Prof. Dr. ____

Instituição: ___________________________ Julgamento:_______________________

Prof. Dr. ____

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Prof. Dr. ____

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Prof. Dr. ____

Instituição: ___________________________ Julgamento:_______________________

“Vinde a mim todos que estais cansados e oprimidos, e eu vos aliviarei

Tomai sobre vós o meu jugo, e aprendei de mim

Que sou manso e humilde de coração e encontrarei descanso para vossas almas

Porque meu jugo é suave e meu fardo é leve”

Mateus 11:28-30

DEDICATÓRIA

Para meus avós Altino e Sebastiana, minha mãe Tania, meu irmão José Roberto e minha afilhada Dandarah.

AGRADECIMENTOS

A Deus pelo dom da vida.

À toda minha família por entender minha ausência e pelo suporte, principalmente durante esta reta final.

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Se em algum momento eu me esqueci que você me ajudou, te peço desculpas. Mas, sou muito grata à você também.

RESUMO

MOREIRA, T. S. **Exigências de energia, partição energética e emissão de metano por novilhas leiteiras Holandês, Gir e F1 Holandês-Gir em crescimento.** [Energy requirements, energetic partition and methane emission from growing Holstein, Gyr and F1 Holstein-Gyr dairy heifers]**.** 2016. 67 f. Tese (Doutorado) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2016.

No Brasil, país com a quinta maior produção mundial de leite, as formulações de dietas ainda são realizadas com base nas exigências nutricionais estabelecidas em países de clima predominantemente temperado. Pesquisas de validação de sistemas nutricionais têm evidenciado incompatibilidade de aplicação dos sistemas gerados em condições temperadas às condições tropicais. Assim, objetivou-se com o presente trabalho determinar as exigências de energia, a partição da energia consumida e emissão de metano (CH_4) entérico de novilhas leiteiras em crescimento das raças Holandês, Gir e F1 Holandês-Gir, em condições tropicais. O experimento foi realizado no Complexo Experimental Multiusuário da Embrapa Gado de Leite, localizado no Campo Experimental José Henrique Bruschi, em Coronel Pacheco – MG. Foram avaliadas 36 novilhas leiteiras, sendo 12 da raça Holandês, 12 da raça Gir e 12 F1 Holandês-Gir em 3 experimentos distintos. No primeiro experimento, as 36 novilhas foram distribuídas em 12 quadrados latinos, em arranjo fatorial 3x3, ou seja, 3 planos nutricionais e 3 grupos genéticos. Os planos nutricionais foram (1) 1.0x mantença; (2) 1.5x mantença e (3) 2.0x mantença e as novilhas foram alimentadas com uma dieta constituída de 85.0% de silagem de milho e 15.0% de concentrado com base na matéria seca (MS). A metodologia empregada para mensuração de CH_4 foi a técnica do gás traçador SF_6. O consumo de matéria seca (CMS) e nutrientes apresentou interação entre genótipo e plano nutricional. Novilhas da raça Gir apresentam maior digestibilidade da proteína bruta (76,55%), as F1 Holandês-Gir valor intermediário (75,14%) enquanto que os animais da raça Holandês apresentaram o menor valor (74,59%). A produção diária de metano em grama dia (g/d) foi influenciada pelo plano nutricional e também diferiu entre grupo genético, sendo que novilhas da raça Gir quando comparadas às demais tiveram menor emissão de CH_4 entérico. Novilhas alimentadas sob o menor plano nutricional apresentaram maior emissão de CH_4 (85,5%) por ganho de peso diário (g/kg de GPD) quando comparada as novilhas sob o maior plano nutricional. A produção média de CH_4 anual encontrada no presente estudo foi de 45,84 kg. O segundo experimento teve como objetivo mensurar a produção de calor (PC) e a emissão de CH_4 entérico por novilhas leiteiras através do método da máscara facial. Os mesmos animais, tratamentos e dietas que foram utilizadas no primeiro estudo foram utilizados neste estudo e no estudo que será descrito posteriormente a esse. A PC expressa em Mcal por peso vivo metabólico (Mcal/PVM) foi afetada por genótipo e novilhas da raça Gir apresentaram menor PC (163,2) quando comparada as novilhas Holandês (201,0) enquanto que as novilhas F1 Holandês-Gir não diferiu das demais (181,3). Observou-se interação entre genótipo e plano nutricional para emissão de CH_4 em grama dia e em grama por quilo de peso vivo metabólico. Quando expresso em relação à matéria seca ingerida, não foram encontrados efeitos de genótipo ou plano nutricional para emissão de CH_4. O terceiro estudo objetivou determinar as exigências de energia, a partição energética e a emissão metano entérico pela metodologia padrão de respirometria calorimétrica. Cada novilha permaneceu por um período de 24 horas no interior da câmara para as mensurações. A emissão de CH_4 (g/d) foi influenciada por genótipo e plano nutricional. Novilhas Holandês e F1 Holandês-Gir demonstram emissões superiores em 73,4% quando comparadas as novilhas da raça Gir. A exigência de energia líquida para mantença (EL_m/kcal $BW^{0,75}$) foi 103,9 para novilhas Holandês, 79,86 para novilhas Gir e 103,8 para novilhas F1 Holandês-Gir. A exigência de energia metabolizável para mantença (EM_m/kcal $BW^{0,75}$) foi 132, 6 para novilhas Holandês, 116,0 para novilhas Gir e 138,2 para novilhas F1 Holandês-Gir. Não foram encontradas diferenças entre novilhas Holandês e F1 Holandês-Gir para exigências de EL_m e EM_m, então foi formulada uma equação combinada para ambas, onde EL_m e EM_m foram 105,2 e 135,0 kcal/$BW^{0,75}$, respectivamente. Concluiu-se que os atuais resultados de exigências em energia tiveram similaridade com a literatura disponível e serão utilizados para inclusão no banco de dados de gado de leite, a ser formado com trabalhos já existentes e outros que ainda serão desenvolvidos, visando ao futuro estabelecimento das normas e padrões nacionais de alimentação para bovinos leiteiros dos grupos genéticos mais representativos do rebanho nacional. Os dados de emissão de metano entérico obtidos poderão ser utilizados na elaboração do inventário nacional de emissão de gases de efeito estufa pelas atividades pecuárias.

Palavras-chave: Aquecimento global. Respirometria. Ruminantes.

ABSTRACT

MOREIRA, T. S. **Energy requirements, energetic partition and methane emission from growing Holstein, Gyr and F1 Holstein-Gyr dairy heifers.** [Exigências de energia, partição energética e emissão de metano por novilhas leiteiras Holandês, Gir e F1 Holandês-Gir em crescimento]. 2016. 67 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2016.

In Brazil, country with the fifth largest world production of milk, diets formulations are also carried out on the basis of nutritional requirements established in other countries, especially those from predominantly temperate climate. Research validation nutritional systems have evidenced application incompatibility of systems generated in temperate conditions at tropical conditions. Thus, the aim of this study was to determinate energy requirements, energetic partition and methane (CH_4) enteric emission from growing Holstein, Gyr and F1 Holstein-Gyr dairy heifers in tropical conditions. The trial was conducted at The Multi-use Livestock Complex of Bioefficiency and Sustainability at Embrapa Dairy Cattle, Coronel Pacheco - MG, Brazil. Were evaluated 36 heifers, 12 Holstein, 12 Gyr and 12 Holstein-Gyr in 3 distinct experiments. In the first one, the 36 heifers were distributed in 12 latin squares, in a 3x3 factorial arrangement which was 3 nutritional plans and 3 genotypes. The nutritional plans were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance and the heifers were fed a diet consisting of 85.0% of corn silage and 15.0% of concentrate on a dry matter (DM) basis. Enteric CH_4 emission was evaluated by SF_6 tracer technique. Dry matter intake (DMI) and nutrients presented interaction among genotype and nutritional plan. Gyr heifers demonstrated higher crude protein (CP) digestibility (76.55%), F1 Holstein-Gyr intermediary value (75.14%) and Holstein animals presented the lowest value (74.59%). Daily CH_4 production (g/d) was influenced by nutritional plan and differed as well between genotypes whereas Gyr heifers compared to the others had lesser CH_4 emissions. Heifers fed at lower nutritional plan presented highest (85.5%) CH_4 emissions by average daily gain (g/ kg of ADG) when compared to heifers fed at the higher nutritional plan. We found annual emissions of 45.84 kg of $CH_{4.}$ The second experiment has as objective measure the heat production (HP) and the enteric CH_4 emission from dairy heifers using face mask (FM) method. The same animals, treatments and diets that were used in the first study were used in this second and third one. The HP expressed in Mcal by metabolic body weight (Mcal/$BW^{0.75}$) was affect by genotype and Gyr heifers presented lower HP (163.2) when compared to Holstein (201.0) while F1 Holstein-Gyr heifers did not differ (181.3). Observed interaction among genotype and nutritional plan to CH_4 emission in (g/d) and grams per kilo of metabolic body weight (g/kg of $BW^{0.75}$). When expressed in dry matter ingested was not found genotype or nutritional plans effects to CH_4 production. In the third study, our objective was to determine energy requirements, energetic partition and enteric CH_4 emission using the "gold standard" methodology as calorimetric respirometry. Each heifers spent one 24 hours period in an open-circuit respirometric chamber (RC) to measurements. The CH_4 emission was influenced by genotype and nutritional plan. Holstein and F1 Holstein-Gyr heifers demonstrated 73.4% superior emissions when compared to Gyr heifers. The net energy requirements for maintenance (NE_m/kcal $BW^{0.75}$) was 103.9 for Holstein heifers, 79.86 for Gyr heifers and 103.8 for F1 Holstein-Gyr heifers. The metabolizable energy requirements for maintenance (ME_m/kcal $BW^{0.75}$) was 132.6 for Holstein heifers, 116.0 for Gyr heifers and 138.2 for F1 Holstein-Gyr heifers. Were not found differences among Holstein and F1 heifers on NE_m and MEm, so was formulated a combined equation for both, where the net and metabolizable energy requirements for maintenance were 105.2 and 135.0 kcal/$BW^{0.75}$, respectively. We concluded that our results about nutritional requirements had similarity with available literature from respirometric chambers. These generated data from dairy cattle will be used for a future data base vising the establishment of feed patterns for representative dairy cattle genotypes in national herd composition. Also, the enteric methane emission data obtained in this study will be used in the greenhouse gases national inventory.

Keywords: Global warming. Resporimetry. Ruminants.

SUMÁRIO

1 GENERAL INTRODUCTION

The growing world population and its acquisitive power has promoted sharp increase in demand for food of animal origin. Projected to increase in world, milk production of 580 million tonnes in 1999-2001 will reach 1,043 million tonnes in 2050 (FAO, 2006). Added to this fact, there is pressure from environmentalists and farmers for sustainable production systems in all aspects (economic, social and environmental), which should contain the advance of cattle on new areas. In this scenario, the increase in productivity in dairy farming will be essential to ensure increases in the production and supply of milk and its derivatives forward to growing demand. Therefore, appropriate technologies should be developed and adopted to enable increased productivity in a sustainable manner, ensuring higher milk production by reducing the number of animals and the occupied area. Noncompliance with the issues may result in trade restrictions in domestic and mainly external. The dairy production occupies a prominent position in Brazilian agribusiness. Milk production reached 36 billion liters in Brazil in 2014, up 2.7% over the previous year (USDA, 2015; IBGE 2015). In this scenario, some technologies developed in the country in the nutritional field have been increasingly adopted, due to its cost-benefit ratio. Nutrition is one of the main factors that affect animal performance and the expenses involved with feeding the animals are very important in milk production systems. Thus, the search and the adoption of more rational measures in the food management have the potential to generate positive economic impact and quality of milk production systems.

The global scientific research in animal nutrition has been defined for more than a century, the animal nutritional requirements (PRESTON, 2006). Knowledge not only the defendants nutrients, as well as the concentration of these in the diet for specific animal category achieve the desired performance, form the basis for the formulation of diets and planning the nutritional management of the herd. Diets able to properly attend the animal nutritional requirements provide the rational use of food and thus reduce the excretion of nutrients in the environment and improve profitability, competitiveness and sustainability of dairy farming (VALADARES FILHO et al., 2005).

Research has been done around the world seeking the best diet adjustment to the nutritional needs of the animal. In livestock developed countries, researchers committees are often formed to gather and organize knowledge in ruminant nutrition for the development of standards and their cattle herds feeding patterns, taking into account the peculiarities of their realities. The formation of these committees allows the best ways to estimate the nutritional requirements and nutritional value of foods are established, guaranteed the guidelines for formulation of maximum bioeconomic efficiency feed, maximizing production and strengthening the competitiveness of production systems.

In Brazil, country with the fifth largest world production of milk, diets formulations are also carried out on the basis of nutritional requirements established in other countries, especially those from predominantly temperate climate. Research validation nutritional systems have evidenced application incompatibility of systems generated in temperate conditions at tropical conditions (LANNA et al., 1994; LANA et al., 2002; BACKES, 2003; VITTORI, 2003; GESUALDI JR, et al. 2005). However, the use of tables and foreign systems (American system NRC, Australian CSIRO, AFRC English and French INRA) is still the option available in Brazil for producers, technicians and researchers.

Technologies to be adopted in Brazil must respect their characteristics in the herd composition, once the available food and climate are typical and unique tropical environments (VALADARES FILHO et al., 2009). The development of a Brazilian database of nutritional requirements and the subsequent development of an accurate model for predicting the nutritional requirements represent an effective alternative to increased productivity and economic efficiency of milk production systems in Brazil.

The determination of nutritional requirements of cattle to different genotypes in Brazilian environmental conditions is important for the consolidation of the country as a leader not only in the production of animal products, but also in the generation of sustainable technologies for animal production in the tropics. Most of the work already carried out has contemplated beef cattle, especially entire males, with lack of information about dairy animals, especially females, including the most used crossing.

In the growing phase, there is pressing the need for establishment weight gain goals and animal nutritional requirements as meeting the nutritional needs effectively and accurately allows the genotype expression and reduction of age of first birth, as well as the reduction of excretion nutrients to the environment as nitrogen, phosphorus, methane, etc. Additionally, unbalanced nutrients diets may also prevent the proper growth, can contrary (energy excess) allowing the high fat deposition and affect the mammary gland development, resulting in negative effects in the first and second lactation and possibly on reproductive performance.

The data generation about energy requirements from dairy cattle and the knowledge of enteric methane emission from this category represent a research effort to meet these demands, seen that few bioenergetics studies were conducted until the present to dairy adaptability in tropical conditions.

In this context, is imperative the research conducting for generate and elaborate the nutritional dairy cattle requirements system, considering the main crossing strategies used in Brazil, involving variable partition of Holstein and Zebu genes.

2 THE EFFECTS OF FEED INTAKE LEVEL ON ENTERIC METHANE EMISSION FROM GROWING DAIRY HEIFERS USING SF_6 TRACER TECHNIQUE

ABSTRACT

The aim of this study was to determinate methane emission using SF_6 tracer technique from growing dairy heifers fed at three different nutritional plans (NP). Thirty-six heifers, 12 Holstein, 12 Gyr and 12 F1 Holstein-Gyr, 10 months old, initial body weight 174.8±42.6 kg, 123.5±44.4 kg and 201.5±44.6 kg to Holstein, Gyr and F1 Holstein-Gyr respectively, were distributed in multiples latin squares in a 3x3 factorial arrangement (3 nutritional plans x 3 genotypes). Within each latin square, heifers were randomly distributed in 3 different nutritional plans (NP) predicted by NRC (2001), which were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance. Heifers were fed a diet consisting of 85.0% corn silage and 15.0% concentrate on dry matter (DM) basis. Dry matter intake (DMI) and nutrients showed interaction among genotype and NP. Gyr heirfers present greatest crude protein (CP) digestibility than other heifers. Average daily gain (ADG) also had interaction between genotype and NP, which was lower to Gyr heifers. Methane emission in grams per day was influenced by intake level and genotype. When heifers increased their intakes, CH_4 emission (g/d) increased and yield (g/kg ADG) declined, and this was associated with a dilution on CH_4 production by live weigh gain. We concluded an important benefit of high feed intake levels is a reduction on emission intensity as CH_4/product.

2.1 INTRODUCTION

The Brazilian livestock has the largest commercial herd in the world, around 89% of beef cattle and 11% of dairy cattle. Due the extensive production systems with large area of degraded grasslands, Brazil has been criticized by highest greenhouses gases emission (GHG). This inefficient scenario generates more amount of GHG by unit of product (IPCC, 2007; MCT, 2010; MACHADO et al., 2011).

Brazilian milk production represents the 5th position in the internationally ranking, behind European Union, India, United States and China (USDA, 2014). The Brazilian herd is composed 70% of crossbred animals, which is frequently crossed with European dairy cattle and Zebu breeds, resulting in F1 Holstein-Gyr cattle. This crossbreeding is to combine the higher milk yields, disease resistance and adaptability to climate conditions. Therefore, resulting in crossbred with dairy merit adapted in tropical conditions. Minas Gerais is the main milk producing state, corresponding for 26% of total milk production and has been improved their production by 14.5% in 2015, compared to previous year (USDA, 2015).

Increasing animal performance by genetic improvement, increasing feed efficiency and decreasing methane per unit of product as meat or milk is some of strategy to reduce the negative impact of livestock production on global warming. (KNAPP et al., 2014).

The aim of this study was to evaluate methane emission from growing dairy heifers fed at three different feed nutritional plans. It was hypothesized that genotypes have no influence on methane emission and the increase on feed intake level would reduce methane emission per unit of daily gain as potential strategy for sustainable ruminant production systems in tropical conditions.

2.2 MATERIAL AND METHODS

This study followed the Ethics Committee in Animal Use of the University of Sao Paulo. The trial was conducted at The Multi-use Livestock Complex of Bioefficiency and Sustainability at Embrapa Dairy Cattle, Coronel Pacheco, MG, Brazil from December 2012 till September 2013.

The experimental design was multiples latin square, in a factorial arrangement 3x3 (3 genotypes and 3 nutritional plans). Within each latin square, thirty six dairy heifers from 3 genotypes (Holstein, Gyr and F1 Holstein-Gyr) were randomly distributed in 3 different nutritional plans (NP) predicted by NRC (2001), which were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance. The experimental unit was the animal within each period. Thus, the experiment had 108 experimental units relating to 36 animals in 3 periods and 12 squares. Each experimental group had 12 animals and in the beginning the initial BW were 123.5±44.4 kg, 174.8±42.6 kg and 201.5±44.6 kg to Gyr, Holstein and F1 Holstein-Gyr, respectively. Heifers were housed in a *tie-stall* system with individuals feed and water bins. The trial started on December 2012, where these heifers completed 10 months old and passed for an adaptation period in the experimental conditions of 80 days. To reduce the variability in the trial, all heifers were contemporary and produced by fixed-time artificial insemination or *in vitro fertilization*. Throughout the present study, heifers diet were total mixed ration (TMR) of corn silage combined with concentrates and the proportion of silage to concentrate was 85:15 based on the dry matter (DM) and the chemical diet composition is described in Table 1. The TMR from 1.0x and 1.5x maintenance treatments were the same composition, differing just in the amount offered to the heifers. Concentrate from 1.0x and 1.5x maintenance treatments were composed of soybean meal (81.4%), urea (7.1%), mineral mix (4.8%), ammonium sulfate (3.5%) and mineral salt (3.2%). Concentrate from 2.0x maintenance was composed of soybean meal (79.1%), ground corn (9.4%), urea (4.4%), mineral mix (2.9%), ammonium sulfate (2.2%) and mineral salt (2.0%). The feed was provided once daily in the morning (08:00h), weighed daily and samples dried to determine DM content, and dry matter intake (DMI) daily was calculated per animal. BW was measured at 7 days intervals and used to adjust the amount of feed offered to each heifer. Each experimental period was 30 d of adaptation to the diets, followed for 8 d of metabolism measurements.

Table 1 - Formulation and chemical composition of experimental diet

Item	Nutritional plan	
	1.0 – 1.5x	2.0x
Formulation	g/kg	
Corn silage	851.0	806.0
Soybean meal	120.0	153.0
Ground corn	0.0	18.0
Urea	11.0	9.0
Ammonium sulfate	5.0	4.0
Mineral mix[a]	8.0	6.0
Mineral salt	5.0	4.0
Total	1000	1000
Composition	g/kg DM	
Dry matter	373.6	373.2
Crude protein	158.9	145.2
Ether extract	31.1	31.1
NDF[b]	456.7	458.9
NFC[c]	287.6	300.4
Energy density	Mcal/kg	
GE[d]	4.39	4.43
ME[e]	2.62	2.73

[a]Guarante levels: Ca:190 g/kg; P:60 g/kg; Na:70 g/kg; Mg: 20 g/kg; Co:15 mg/kg; Cu:700 mg/kg; Mn:1.600 mg/kg; Se:19 mg/kg; Zn:2.500 mg/kg e I:40 mg/kg.
[b]Neutral detergent fiber; [c]Non-fibrous carbohydrates; [d]gross energy
[e]Metabolizable energy determined in metabolism trial

2.2.1 Dry matter intake and digestibility

Digestibility trials were conducted at three periods throughout the experiment: March, June and September. Voluntary intake was calculated by feed provided during five consecutive days of each experimental period. Ingredients of the concentrate were collected for analysis and representative samples of silage, concentrate and orts were collected daily and pooled for chemical analysis. Samples were analyzed using Association of Official Analytical Chemists (AOAC 1995) methods for analytical DM, OM (method 930.15), CP (method 990.03) and EE (method 920.39). Gross energy was determined using an adiabatic calorimeter (model C-5000, Labcontrol IKA, São Paulo, SP). Acid detergent fiber was determined according to Van Soest et al. (1991). Neutral detergent fiber was determined according to Van Soest et al. (1991) with a heat stable amylase and expressed exclusive of residual ash.

Total feces were collected for three consecutive days from all animals at all periods. At the end of each collection day, feces of each animal were weighed. The feces were sampled after homogenization. The samples were weighed, dried in a forced-ventilation oven (55^{o}C) for 72 h, and ground through a 1 mm screen (Wiley mill; A. H. Thomas, Philadelphia, PA). One composite sample per animal, based on the DM weight for every collection day was prepared for chemical analysis. The same chemical analyses that were performed for experimental diet were also performed for feces to calculate the DM, nutrients and energy digestibility coefficients.

2.2.2 Lipidic metabolism

Blood samples were collected from coccygeal vein on day 3 of digestibility trial and immediately centrifuged at 5000 rpm for 15 minutes at room temperature. Samples were stored at -20ºC until analyzed. Non-esterified fatty acids (NEFA) (Randox Laboratories, Ltd, UK, Cat # FA 115) concentrations were determined spectrophotometrically (Shimadzu UV 1601).

2.2.3 Animal performance

All weighing were made in the beginning and in the end of each experimental period, in the same time, at 08:00 h in the morning immediately before feeding. The average daily gain (ADG) was determined by the difference between the final and the beginning body weight divided by number of days of the experimental period. Feed efficiency (FE) was calculated conform this equation: FE = ADG (kg DM/d)/DMI (kg/d).

2.2.4 Methane emission measurement by SF_6 tracer technique

The SF_6 tracer gas technique (JOHNSON et al. 1994) was used to estimate daily CH_4 emissions. The SF_6 release rate and expected lifetime of permeation tubes was calculated using prefilling weight of SF_6 within the tube and serial change in weight over 11 wk within a controlled environment at 39^{o}C. The release rates of SF_6 tubes ranged from 1.40 to 1.85 mg/d, with a mean of 1.66 ± 0.147 mg/d, and lifespans of 330 ± 119 d. A permeation tube containing SF_6 gas of known release rate was orally inserted into the rumen of each heifer using a stomach tube the week before measurements were made. After 5 wk of adaptation, CH_4 collection was initiated for a period of 1 h before feeding (0730 h) for a period of 24 h, with the procedure repeated on 5 consecutive d. Expired CH_4 and SF_6 was collected by placing a head collar on each heifer that possed a gas collection tube that ran from just above the animal´s nostrils to an evacuated gas canister (-15 PSI). Background concentrations of SF_6 and CH_4 were measured daily by hanging two evacuated canisters at either end of the *tie-stall* barn. Canisters were made of polyvinyl chloride (PVC) equipped with a capillary tube (0.127-mm diameter) that was used to sample gas with the vacuum inside the canister remaining at 40-60% of the initial vacuum after 24 h of measurement. After 24 h, canisters containing samples of SF_6 and CH_4 were removed from heifers, gas was sampled and the pressure was recorded. Background canisters were collected for analysis at the same time as canisters were collected from heifers. If the pressure inside the canisters was below or above the 40-60% range, gas samples were not collected, and an additional CH_4 measurement day was added to ensure that at least 5 d of CH_4 measurement were collected from each animal. Gas samples from each canister (20 mL) were collected and placed into 5 pre-evacuated 12 mL Exetainers (Labco Limited, Lampeter, UK). The SF_6 (ppt) and CH_4 (ppm) concentrations in the sampling canisters were determined using two separate gas chromatographs; models 6890N plus and 7820A, respectively (Agilent Technologies, Santa Clara, CA). Both chromatographs were equipped with a split-splitless injector, but a μECD detector (electron capture) was used to measure SF_6 and a FID detector (flame ionization) was used to measure CH_4 concentration.

For SF_6 analysis, a 30 m × 0.530 mm × 25.0 μm column (HP-Molsieve, Agilent Technologies, Santa Clara, CA) was used with N_2 as carrier gas at a flow rate of 5.0 mL/min with N_2 as the makeup gas at 40 mL/min. The μECD detector was maintained at 300ºC and N_2 at 40 mL/min was used as the carrier gas. Oven temperature was kept at 50^{o}C for 4 min to elute the desired constituents. The gas chromatograph was calibrated weekly using SF6 (White Martins, São Cristóvão, RJ) standards ranging in concentrations from 30, 100, 500, 1500, 3000 ppt. The CH_4 was analyzed using two columns, a 30 m × 0.530 mm × 40.0 μm column (HP-Plot/Q, Agilent Technologies, Santa Clara, CA) and a 30 m × 0.530 mm × 25.0 μm column (HP-Molsieve, Agilent Technologies, Santa Clara, CA) with H_2 as carrier gas at a flow rate of 7.0 mL/min. The FID detector was maintained at 280ºC, 10 mL/min of H_2 flow, 400 mL/min of synthetic air, and 20 mL/min of complementation flow. Oven temperature was kept at 50ºC for 4.5 min to elute the desired constituents. The gas chromatograph was calibrated using CH_4 (Linde AG, Rio de Janeiro, RJ) at 4.8, 9.7, 19.6, 102, 203 ppm. Emission rate of enteric CH_4 (ECH_4; g/animal/d) was calculated from the measured SF_6 and CH_4 concentrations sampled from the canisters ($[CH_4]M$; ppm and $[SF_6]M$; ppt), the background SF_6 and CH_4 concentrations ($[CH_4]BG$; ppm and $[SF_6]BG$; ppt), the molecular mass of CH_4 ($MWCH_4$ = 16) and SF_6 ($MWSF_6$ = 146) and the predetermined release rate of the permeation tubes (RSF_6; mg/d) as described by Williams et al. (2011):

$$ECH_4 = RSF \; \frac{CH_4\,M - CH_4\,BG}{SF_6\,M - SF_6\,BG} \; x \left(\frac{MW\;CH_4}{MW\;SF_6} \right) x \; 1000$$

2.2.5 Statistical analysis

Data were analysed by Statistical Analysis System being previously verify the normality of residuals by the Shapiro-Wilk test and the homogeneity of variances compared by the Levene test. These data were submitted to the analysis of variance, using the mixed models procedure (PROC MIXED), which causes of variation contemplated intake level effect, genotype and the interaction as fixed effects, as well as animal nested inside the square, squares and periods as random effects. The intake level effect was evaluated by using polynomial regression, separating the linear and deviation from linearity effects. The genotype effect was evaluated using the Tukey test at 0.05 significance.

2.3. RESULTS AND DISCUSSION

The dry matter and nutrients intake (kg/d) are presenting in Table 2. There was a significant interaction among factors (genotype and nutritional plan) to all nutrients. When expressed in g/kg of $BW^{0.75}$, DM and OM had interaction among genotype and nutritional plan. DM and nutrients intake was lower ($p<0.05$) to Gyr heifers compared to the others but Holstein and F1 Holstein-Gyr did not differ ($p>0.05$). In relation to intake level, except to NDF, DM and others nutrients had a linear effect, increasing according the NP.

Table 2 - Nutrients intake (kg) of growing dairy heifers

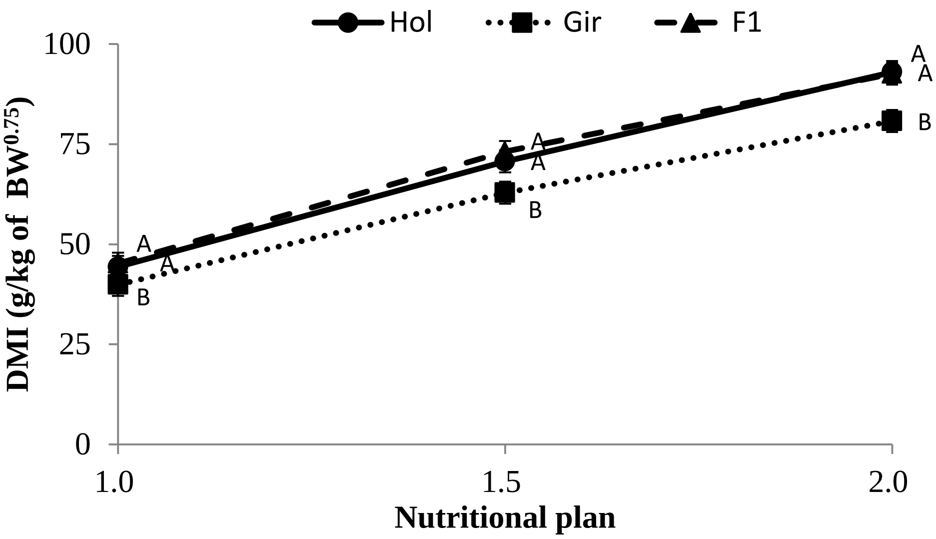
Variable	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gir	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
DM	4.43^{a}	3.10^{b}	4.92^{a}	2.78	4.21	5.61	4.21	0.17	<.0001	$<.0001_{L}$	<.0001
CP	0.74^{a}	0.51^{b}	0.81^{a}	0.46	0.70	0.93	0.70	0.03	<.0001	$<.0001_{L}$	0.0011
NDF	1.98^{a}	1.39^{b}	2.19^{a}	1.27	1.91	2.44	1.88	0.07	<.0001	$<.0001_{L}$	0.0007
ADF	1.24^{a}	0.88^{b}	1.38^{a}	0.80	1.20	1.53	1.18	0.05	<.0001	$<.0001_{L}$	0.0052
EE	0.14^{a}	0.10^{b}	0.15^{a}	0.09	0.13	0.17	0.13	0.005	<.0001	$<.0001_{L}$	0.0498
OM	4.13^{a}	2.90^{b}	4.59^{a}	2.60	3.93	5.22	3.93	0.16	<.0001	$<.0001_{L}$	<.0001
			g/kg $BW^{0.75}$								
DM	69.35^{a}	61.18^{b}	70.29^{a}	43.29	69.18	89.11	67.19	1.95	<.0001	$<.0001_{L}$	0.0030
OM	64.65^{a}	57.05^{b}	65.54^{a}	40.39	64.54	83.00	62.64	1.81	<.0001	$<.0001_{D}$	0.0019
NDF	30.90^{a}	27.35^{b}	31.29^{a}	19.66	31.34	38.88	29.96	0.84	<.0001	$<.0001_{D}$	NS

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype.
Means with different letters within a row differ significantly according Tukey test, p<0.05

The prediction of DMI in heifers is a decisive aspect in nutrition programs and published data of dairy heifers DMI is sparse (HOFFMAN et al., 2008). Accurately database of dairy heifers DMI across genetic groups have not been available in tropical regions. In current study, DMI (g/kg of $BW^{0.75}$) interaction (Figure **1**) show that Gyr heifers had the lowest ($p<0.05$) intake in all treatments, representing 12.1% less compared to Holstein and F1-Holstein-Gyr. Increasing intake level this difference is clearly and strongly demonstrated, confirming the reduced digestive capacity from *Bos taurus indicus*, independently of nutritional plan. Supporting our results, published data from Ferrell and Jenkins (1998) showed that *Bos taurus indicus* presented lesser digestive tract weight than *Bos taurus taurus*. In other studies, some authors reported that *Bos taurus taurus* had greater intake than *Bos taurus indicus* when fed good or medium quality diets and this difference on performance between genotypes is related when rumen function is not limited by nutrient deficit (HUNTER and SIEBERT 1985; FRISH and VERCOE 1977). Rennó et al. (2005) comparing effects of dietary urea levels on DMI across Holstein, Zebu and crossbred (Holstein-Guzera and Holstein-Gyr) steers reported interaction ($p<0.05$) among treatments, also finding differences on intake where Zebu ingested less than Holstein. Evaluating equations to predict DMI in Holstein and crossbred Holstein-Jersey from 4 mo of age until 5 weeks prepartum

Hoffman et al. (2008) found interaction among breed and body weight and also reported differences on DMI models, where NRC (2001) underpredicted DMI of light Holstein and crossbred heifers and conversely overpredicted DMI of heavy heifers. The authors claimed that this was because Holstein and crossbred heifers differ in mature size potential and their metabolic efficiency at similar BW. Previous studies showed that level of nutrient intake during peripubertal period on heifers could have long lasting effects on productivity, profitability and reproduction (DANIELS et al., 2009; PIANTONI et al., 2012). Few studies evaluated the nutrition influence on reproductive performance. Recently, Sartori et al. (2016) reported that effects of feed intake on reproduction might differ between Holstein and Nellore cows due insulin and hormones concentrations. Overall, unbalanced diets can make negatives effects on mammary gland development, affecting first and second lactation.

Figure 1 - Effect of interaction between genotype and nutritional plan to DMI g/kg of $BW^{0.75}$



Overall, greater intake levels can contribute to reduce nutrients digestibility (NRC, 2001), in present study was not find any effect of interaction (p>0.05) among genotype and nutritional plan to nutrients digestibility. However, Gyr heifers had greatest (p<0.05) CP digestibility (Table 3) in comparison to the others genotypes. Some studies have shown differences on the nutrients digestibility between *Bos taurus taurus*, *Bos taurus inducus* and crossbred. These differences are related to the higher capacity of zebu animals to digest nutrients, especially when feeding diets of poor quality (rich on fiber). In the present study, it was not this case, but the intake difference among genotypes, which was lower to Gyr heifers could explain this greatest CP digestibility due the digest retention time, contributing to maximize the digestibility. In the other hand, this found could be explained due the smaller grastintestinal tract from zebu animals, resulting in less dry matter intake as reported by Ferrell and Jenkins (1998). Gonçalves et al. (1991) evaluated intake and digestibility nutrients from Nellore, Holstein and crossbred, and also found elevation on digestibility (dry matter and energy) when reduced feed was offered.

Table 3 - Nutrients digestibility (%) of growing heifers

Item	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
DM	70.42	70.82	69.53	70.11	69.83	70.73	70.23	0.31	NS	NS	NS
OM	71.78	72.53	71.13	71.71	71.45	72.17	71.78	0.30	NS	NS	NS
CP	74.59^{b}	76.55^{a}	75.14^{ab}	75.53	75.16	75.46	75.39	0.36	0.0315	NS	NS
NDF	56.92	58.27	56.31	57.95	56.70	56.71	57.12	0.51	NS	NS	NS
ADF	58.67	59.58	58.54	59.70	58.29	58.71	58.90	0.53	NS	NS	NS
EE	82.22	82.03	81.90	82.19	83.49	80.44	82.04	0.65	NS	NS	NS

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype.
Means with different letters within a row differ significantly according Tukey test, p<0.05

Summary statistics for performance are presenting on Table 4. Feed efficiency (FE) was higher ($p<0.05$) for Holstein compared to Gyr heifers, and F1 Holstein-Gir heifers did not differ to the others. Many metabolic factors can affect the feed efficiency in cattle, including behavior, feed intake, level of production, digestion and digestibility (WAGHORN and DEWHURST, 2007; HERD and ARTHUR, 2009). In the other hand, Willians at al. (2011) evaluating variation on residual feed intake from Holstein-Friesian dairy heifers reported that the effects of feeding level on efficiency are not well understood, suggesting that the feeding level is usually variable over in a season in pasture based system.

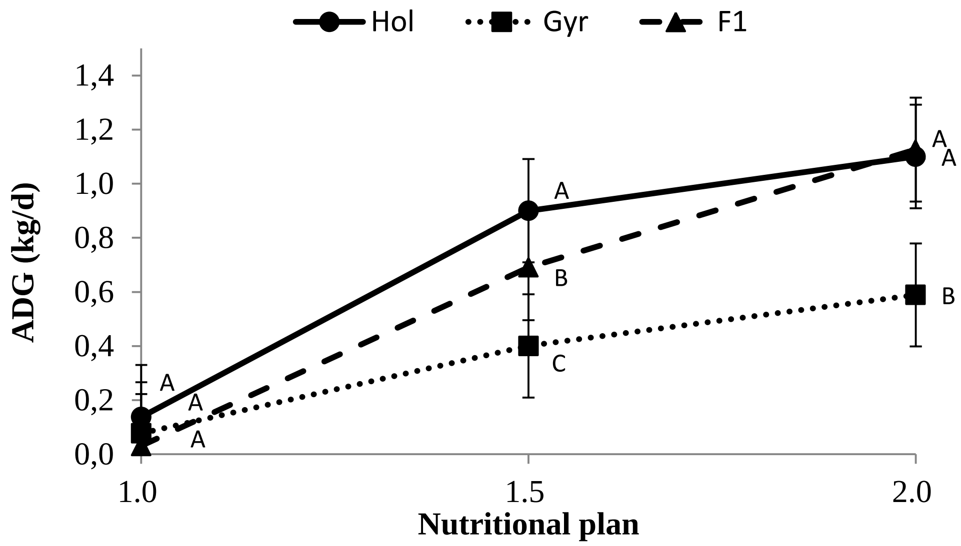
Table 4 - Performance traits of growing heifers

Variable	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
FE	0.18[a]	0.14[b]	0.16[ab]	-	0.15	0.16	0.16	0.006	0.0221	NS	NS
ADG kg/d	0.73[a]	0.34[b]	0.58[a]	0.07	0.65	0.94	0.54	0.06	<.0001	$<.0001_L$	<.0001

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype; FC: feed conversion; FE: feed efficiency;
Means with different letters within a row differ significantly according Tukey test, $p<0.05$

Average daily gain (ADG) presented interaction ($p<0.01$) among factors (Figure 2). In the 1.0 maintenance, heifers of different genotypes did not differ ($p>0.05$). However, in the intermediary and higher intake, Gyr heifers presented a difference on ADG corresponding 48.3% when compared to Holstein and F1 Holstein-Gyr. Pancoti (2015) also reported differences on ADG from Gyr heifers compared to Holstein, 0.63 *vs.* 0.95, respectively. In both studies, these were related to the DMI, superior in Holstein heifers.

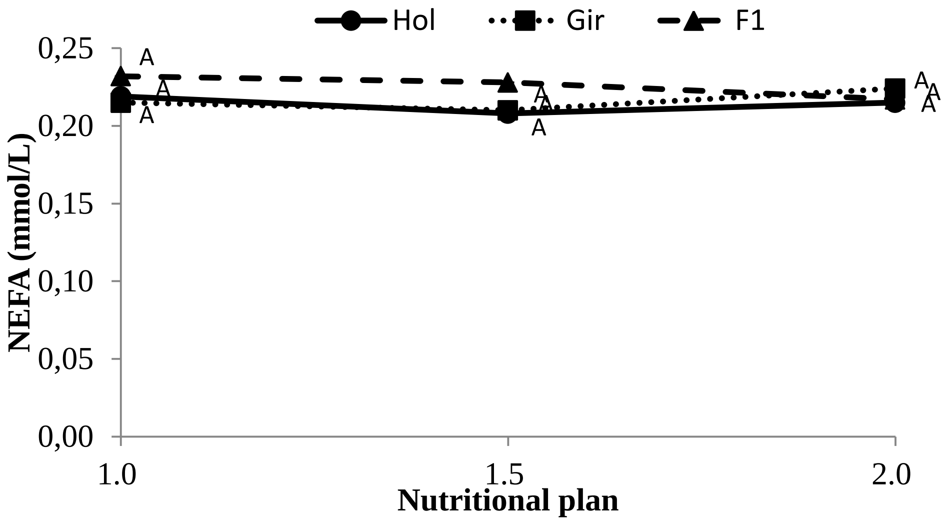
Figure 2 - Effect of interaction between genotype and nutritional plan to ADG (kg/d)



In the present study was not found genotype, nutritional plan or interaction effects to non-esterified fatty acids concentration (Figure 3).

When energy needs are not adequately attending, lipids mobilization from adipose tissue and development of negative energy balance (NEB) are observed, especially on transition period. The NEB response is followed by NEFA blood elevation or hormone changes (GRUMMER, 2008). In the growing phase, there is the need for meet nutritional requirements, otherwise, diets below of requirements may results in negative effects during this period. Some authors reported that NEFA important indicator of body reserves mobilization (DUFFIELD and LEBLANC 2009; LEBLANC, 2010). Available literature from dairy cattle suggests reference values ranging from 0.3 - 0.7 mmol/L, to prepartum and postpartum, respectively (OSPINA et al., 2010). As was not find differences (p>0.05) on NEFA concentrations, which was 0.22 mmol/L on average our results supports that heifers fed at maintenance level has not presents lipid mobilization.

Figure 3 - Non-esterified fatty acids (NEFA) of growing dairy heifers



Methane emission (g/d) was influenced ($p<0.05$) by intake level with a linear response (Table 5). Heifers fed at highest intake presented superior emission of 55.5% and 39.2% in comparison to 1.0 and 1.5 maintenance levels, respectively. To this variable, it was also found an effect ($p=0.0151$) of genotype, with lower emission to Gyr heifers. The lower CH_4 for Gyr genotype can be attributed to DMI which was lowest for these heifers. Overall, the hypothesis that CH_4 emission in g/d would be increased by intake level was supported in the present study. Hammond et al. (2014) reported linear increase in CH_4 production (13.1 - 31.9 g/d) when sheep fed fresh perennial ryegrass were submitted to five different feed intakes. Published data from open-circuit respiration chamber (JONKER et al., 2016 no prelo)[1] show that growing dairy Holstein-Friesian x Jersey heifers with similar body weight (121±6 kg) fed ad libitum fresh pasture emitted 64g of CH_4/d, 59.5% lower than observed at present study, worth pointing out is that the technique and experimental conditions, tropical *vs*. temperate climate were different. This study confirmed the relationship between intake level and CH_4 production as previously observed by Blaxter and Claperton (1965).

When expressed in g/kg of $BW^{0.75}$, Mcal/d and kg/year it was found linear ($p<0.05$) effect of nutritional plan.

[1] JONKER, A.; MOLANO, G.; KOOLAARD, J.; MUETZEL, S. Methane emissions from lactating and non-lactating dairy cows and growing cattle fed fresh pasture. **Animal Production Science**, 2016. No prelo.

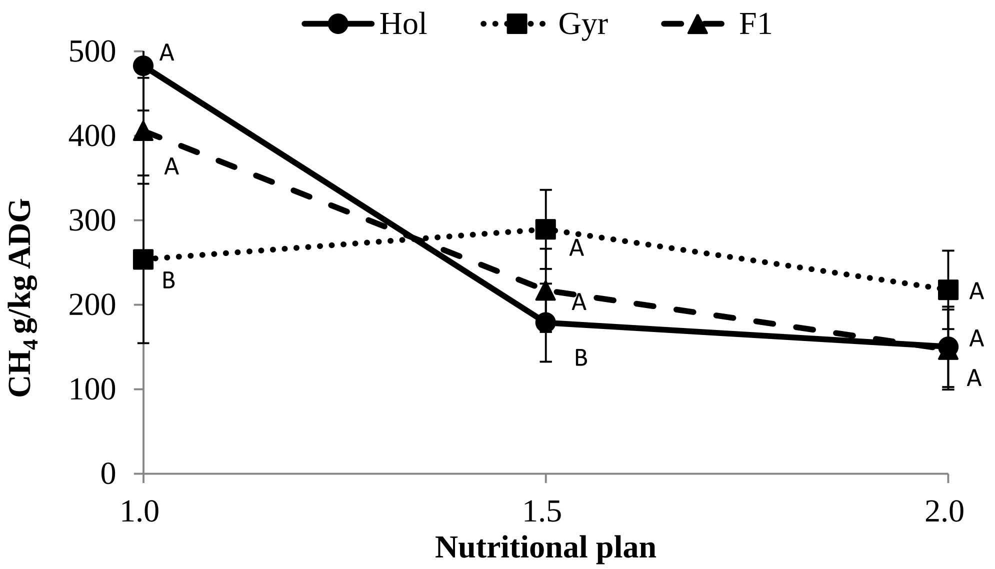
Table 5 - Methane emission of growing heifers

Item	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
g/d	136.78[a]	104.67[b]	139.40[a]	103.75	115.86	161.30	125.58	6.17	0.0151	<.0001$_L$	NS
g/kg DMI	33.64[ab]	38.22[a]	30.78[b]	40.20	30.81	31.36	34.49	1.30	0.0636	0.0017$_L$	NS
g/kg $BW^{0.75}$	2.48	2.04	2.30	1.82	1.90	3.05	2.27	0.15	NS	0.0009$_L$	NS
g/kg ADG	223.45	247.16	194.46	341.18	228.54	183.90	222.70	15.71	NS	<.0001$_L$	0.0049
Mcal/d	2.39	1.64	2.29	1.58	1.84	2.85	2.09	0.20	NS	0.0002$_L$	NS
kg/year	49.92[a]	38.20[b]	50.88[a]	37.87	42.30	58.80	45.84	2.25	0.0151	<.0001$_L$	NS

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype; g/kg $BW^{0.75}$: grams per kg of metabolic body weight;
Means with different letters within a row differ significantly according Tukey test, p<0.05

The important way to comparing greenhouse gas emission of livestock including many feeding systems is CH_4 production per unit of animal product (KURIHARA et al., 1999). In this study it was found interaction (Figure 4) among genotype and nutritional plan to CH_4 emission by unit of daily gain (g/kg of ADG).. At the 1.0x maintenance nutritional plan, CH_4 production/kg of ADG was 90.4% and 60.0% higher for Holstein and F1 Holstein-Gyr when compared to Gyr genotype, respectively. However, at the 1.5x maintenance nutritional plan, CH_4 production/kg of ADG presented by Gyr genotype was 61.7% bigger when compared to Holstein genotype, with no difference among genotypes at 2.0x maintenance nutritional plan. Looking that interaction under other perspective, Holstein and F1 genotypes had decreased CH_4 production/kg of ADG with increasing levels of nutritional plan, resulting in expected improvement of system efficiency. However, interesting to note that this same phenomena did not happen with Gyr genotype, where CH_4 production/kg of ADG was almost constant all over the different nutritional plans used here. So, Gyr genotype does not respond with increasing efficiency when nutritional plan is also improved. Our objective with this 1.0x maintenance nutritional plan would reflect degraded grasslands during the dry season when the poor grass quality available does not attend the maintenance requirements, thereby showing this inefficiency system (Figure 4). Usually, on typical commercial dairy farms in Brazil, the majority diets are corn silage in the dry season and pasture predominantly *Brachiaria* during wet season. The apparent relationship between ADG and CH_4 emission in current study is in agreement with Richmond et al. (2014) who found that Holstein-Friesian steers, Charolais crossbred steers and Charolais crossbred heifers emitted 25% lower CH_4 expressed in g/kg of ADG when grazing improved *vs*. semi-natural grasslands. A study conducted by Boland et al. (2014) which Limousin cross beef heifers performance was measured, these authors demonstrated increased ADG and reduced CH_4 emissions per kilogram of live weigh gain when pregrazing low herbage mass in comparison high herbage mass, 135 *vs*. 165 g/kg of CH_4/ADG respectively. In this context, to represent tropical regions, Kurihara et al. (1999) evaluating CH_4 production and energy partition from Brahman heifers under different diets observed that heifers fed immature Rhodes grass hay (*Chloris gayana*) presented CH_4 emission 3.9 fold higher than those fed Lucerne (*Medicago sativa*) hay plus high-grain diet. These results also demonstrated that relationship between CH_4 emission and live weight gain differ when heifers fed tropical forages from those fed diets based on temperate forages.

Figure 4 - Effect of interaction between genotype and nutritional plan to CH_4 g/kg of ADG



Previous studies showed that increasing animal productivity has a decrease in CH_4 yield as litter of milk or beef produced (PINARES PATIÑO et al., 2009; CLARK, 2013), according with findings in present study. The improvement of livestock production by improving diet quality, livestock management, genetic potential especially in developing countries is an important way to reduce CH_4 production per unit of animal products (SHIBATA et al., 2010).

When evaluated CH_4 emission per year, was found 45.84 kg of CH_4 on average, with linear increase due nutritional plan and it was also found genotype effect ($p<0.05$). Pioneers studies with SF_6 technique in Brazilian tropical conditions, Primavesi et al. (2004) reported annual emissions from dairy heifers ranging from 66-81 kg of CH_4/year and 121-147 kg of CH_4/year to dairy cows. These findings are superior that those described for North America and Europe dairy cows, 118 and 110 kg of CH_4/year, respectively. In current study this reduced annual emission can be explained due the body weight and age presented from these growing heifers. Although many techniques available to measure and generate data about GHG emissions, the IPCC (2006) reported the importance of specific informations as animal category, diet composition, enteric fermentation products composition and mitigate strategies on GHG inventories.

2.4 CONCLUSIONS

The feed intake levels proposed showed positive effects on enteric methane emission. When heifers increased their *TMR* intakes, CH_4 emission (g/d) increased but CH_4 emission/kg of ADG declined, and this was associated with a dilution on CH_4 production by daily gain. However, this happens for some genotypes, but not for others, like Gyr genotype. This strong relationship between CH_4 emission and ADG we concluded an important benefit of high feed intake levels is a reduction on emission intensity as CH_4/product and this finding could be useful to GHG decreasing in Brazil.

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3 HEAT PRODUCTION AND METHANE EMISSION MEASURED BY FACE MASK METHOD IN GROWING DAIRY HEIFERS

ABSTRACT

The aim of this study were to measure heat production (HP) and methane emission (CH_4) from growing dairy heifers fed at different nutritional plans (NP). Thirty-six heifers, 12 Holstein, 12 Gyr and 12 F1 Holstein-Gyr, 17 months old, initial body weight 283.8±50.2 kg; 233.9±55.8 kg and 323.2±59.7 kg from Holstein, Gyr and F1 Holstein x Gyr, respectively, were distributed into 2 blocks in a 3x3 factorial arrangement (3 nutritional plans x 3 genotypes). Heifers were randomly distributed in 3 different nutritional plans (NP), which were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance. Heifers were fed a diet consisting of 85.0% corn silage and 15.0% concentrate on dry matter (DM) basis. The HP and CH_4 were evaluated using a face mask method (FM). Dry matter (3.88 kg/d) intake (DMI) and metabolizable energy (9.41 kg/d) intake (MEI) were lower to Gyr heirfers compared to the others. Body weight (BW) was affect by genotype once Gyr heifers also had less (240.2 kg) BW in comparison F1 Holstein-Gyr. The HP expressed in Mcal by metabolizable body weight (Mcal/$BW^{0.75}$) was also effect by genotype and Gyr heifers present less (163.2) compared to Holstein (201.0) and F1 Holstein-Gyr did not differ (181.3). An interaction among genotype and nutritional plan were found to CH_4 emission g/d and g/kg of $BW^{0.75}$. When presented in CH_4 yield (CH_4 in g/kg DMI) it was not find differences ($p>0.05$) among genotypes and intake level, indicating that CH_4 production is related to diet composition and quality.

3.1 INTRODUCTION

Heat production (HP) is a major element overall of growing animals (ARIELI et al., 2002). It can be measured by respiration chamber (RC) designed as “gold standard” technique which measures oxygen consumption, carbon dioxide and methane production. The RC methodology do not represent the animals in farm conditions. May thus sub or super-estimate the HP, consequently affecting the energy requirements according to NRC (1996). The face mask (FM) system is an alternative technique developed to reduce the stress on the animal and allows oxygen consumption measurements in a natural environment (TAYLOR, 1982). Brosh et al. (1998) and Aharoni et al. (2003) have shown that measuring oxygen consumption per heart beat (oxygen pulse: O_2P) over a short period of time (20 min) is representative of daily O_2P. Since oxygen is carried throughout the body by circulatory system, it has been shown that heart rate measurements are predictive of oxygen consumption and therefore heat production (BOOYENS and HERVEY, 1960; WEBSTER, 1967; YAMAMOTO et al., 1979). FM system has also been reported as alternative methane emission method in ruminants (JOHNSON and JOHNSON, 1995; BHATTA et al., 2007; OSS et al., 2016).

Identifying energy expenditure from energetic metabolism in growing cattle should provide many food efficiency utilization informations, also, differences in different genotypes on body size, digestive capacity, feeding patterns and animal productivity may play some influence on HP in cattle. In addition, informations about energetic partition from Zebu cattle and their crossing are sparse in literature. Maximizing animal production and identifying efficient genotypes can contribute sustainable animal production in the tropics regions. Given these reasons, the objectives of this experiment were to measure heat production and methane emission (CH_4) from growing dairy heifers fed at different nutritional plans.

3.2 MATERIAL AND METHODS

This study followed the Ethics Committee in Animal Use of the University of Sao Paulo. The trial was conducted at The Multi-use Livestock Complex of Bioefficiency and Sustainability at Embrapa Dairy Cattle, Coronel Pacheco, MG, Brazil from July to August 2013.

Animals were allocated into two blocks and treatments tested in the present study were organized in a factorial alignment with 3 nutritional plans and 3 genotypes. Thirty six dairy heifers from 3 genotypes (Holstein, Gyr and F1 Holstein-Gyr) were randomly subdivided in different nutritional plans predicted by NRC (2001), which were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance. Starting aged approximately 17 months, body weight of 283.8±50.2 kg; 233.9±55.8 kg and 323.2±59.7 kg from Holstein, Gyr and F1 Holstein- Gyr, respectively. Throughout the present study, heifers diet were total mixed ration

(*TMR*) of corn silage combined with concentrates and the proportion between silage and concentrate was 85:15 based on the dry matter (DM) and the diet chemical composition is described in Table 1. The *TMR* from 1.0x and 1.5x maintenance nutritional plans had the same composition, differing just in the amount offered to the heifers. Concentrate from 1.0x and 1.5x maintenance nutritional plans were composed of soybean meal (81.4%), urea (7.1%), mineral mix (4.8%), ammonium sulfate (3.5%) and mineral salt (3.2%). Concentrate from 2.0x maintenance nutritional plan was composed of soybean meal (79.1%), ground corn (9.4%), urea (4.4%), mineral mix (2.9%), ammonium sulfate (2.2%) and mineral salt (2.0%). The feed was provided once daily in the morning (08:00 h), weighed daily and samples dried to determine DM content and dry matter intake (DMI) daily was calculated per animal (Table 2).

Table 1 - Formulation and chemical composition of experimental diet

Item	Nutritional plan	
	1.0 and 1.5x	2.0x
Formulation	g/kg	
Corn silage	851.0	806.0
Soybean meal	120.0	153.0
Ground corn	0.0	18.0
Urea	11.0	9.0
Ammonium sulfate	5.0	4.0
Mineral mix[a]	8.0	6.0
Mineral salt	5.0	4.0
Total	1000	1000
Composition	g/kg DM	
Dry matter	356.7	356.6
Crude protein	166.1	150.4
Ether extract	38.1	38.4
NDF[b]	472.6	473.3
NFC[c]	254.1	268.5
Energy density	Mcal/kg	
GE[d]	4.43	4.46
ME[e]	2.62	2.73

[a]Guarante levels: Ca:190 g/kg; P:60 g/kg; Na:70 g/kg; Mg: 20 g/kg; Co:15 mg/kg; Cu:700 mg/kg; Mn:1.600 mg/kg; Se:19 mg/kg; Zn:2.500 mg/kg e I:40 mg/kg.
[b]Neutral detergent fiber;
[c]Non-fibrous carbohydrates;
[d]gross energy
[e]Metabolizable energy determined in metabolism trial

Before starting FM measurements, heifers were adapted for 4 weeks to the diet and were trained to use the face mask for 3 d by placing each heifer in a squeeze chute for 20 min with the face mask attached to their face. Following the adaptation period, three oxygen pulse (O_2P; mL heart beat) measurements were collected over 3 d, separated by 3 d of heat rate (HR; rate/min) measurements at the tie stall. Heart rate (HR; beats/min) was recorded with Polar equine transmitter and monitor (Model S610i; Polar Electro Inc., Kempele, Finland). The transmitter and monitor were embedded in a 10 cm wide elastic girth strap with a velcro latch, that was placed around the animal's girth immediately behind the front shoulders. The negative electrode was positioned about 15 cm to the right of the midline and the positive electrode was positioned on the opposite side of the heifer, parallel to the left elbow. The areas around the electrodes were shaved and conductivity gel applied to increase conductance. Heart rate measurements during the oxygen consumption and during the 3 days of HR at the tie stall were averaged and recorded every 60 sec. Any time during the 3 days of HR measurements that the heifers were disturbed or removed from the tie stall, HR data was discarded.

Oxygen consumption and methane emission data was recorded with the Sable System (Sable Systems International, Las Vegas, NV, USA) attached to the face mask. The air flow rate (standard temperature and pressure; STP) through the mask (100 L/min) was controlled and measured by a mass flow controller (Flow Kit 500H; Sable Systems International, Las Vegas, NV, USA) and set to keep the CO_2 concentration in the mask at 0.8% and based in metabolizable body weight. The mass flow controller acquired sub-samples of air from the mask at 500 mL/min for analysis at the same time as a positive pressure pump (B-pump, Sable Systems, Henderson, NV) acquired sub-samples of ambient air (baseline) at 500 mL/min. Both gas samples from the FM and the ambient air were continuously sampled through Bev-A-Line tubes and a gas switching system (RM-8 Flow Multiplexer, Sable Systems, Henderson, NV) so as to deliver gas samples to the analyzer set by a means of a diaphragm subsampling pump (SS-4 Sub-Sampler Pump) at 200 mL/min.

During 1 min intervals over 20 min, samples from the FM were collected, with ambient air collected over 5 min before and after the 20 min measurement to establish baseline gas levels. All data were recorded with an automated data acquisition program (Distributed MR v2.2; Sable Systems; Henderson, NV). Oxygen consumption (VO_2; mL/min) was calculated from the product of the standard flow rate (STDfr) and the difference in the average from the FM (O_2 fm% and baseline O_2b; %, O_2 concentrations measurements over 20 min as follow:

$$VO_2 = [STDfr \ x \ (O_2fm - O_2b)]$$

Oxygen pulse (O_2P; mL/heart beat) was determined by the average oxygen consumption per min over the average HR per min during the same 20 min period. Total daily oxygen consumption (L/d) was calculated from the product of the average of O_2P and average daily HR. Daily heat production (HP) was then calculated as the product of total daily oxygen consumption and the constant 20.47 kilojoules per liter of oxygen (Nicol and Young, 1990).

Gas measurements were corrected for differences in humidity, lag time and drift, and CH_4 emission (mL/min) for each period was estimated. The CH_4 analyzer (zero and span) was calibarated daily, before measurements and nitrogen gas (98.996%) was used to zero the analyzer. The CH_4 was spanned by using a mixed gas (1.004% CH_4 using N_2 as a carrier).

The CH_4 emission (VCH_4; mL/min) was calculated from the product of the standard flow rate (STDfr) and the difference in the average of the FM (CH_4fm; % and baseline (CH_4b; %); CH_4 concentrations measurements over 20 min) as follows:

$$VCH_4 = STDfr \ x \ CH_4fm - CH_4b$$

VCH_4 (mL/min) was extrapolated to 24 h by multiplying by 1.44 (1440/1000) to convert to L/d and then converted to g/d (1 gCH_4 = 1.4 L CH_4).

3.2.1 Statistical analysis

Data were analysed by Statistical Analysis System, being previously verifying the normality of residuals by the Shapiro-Wilk test and the homogeneity of variances compared by the Levene test. These data were submitted to the analysis of variance, using the mixed models procedure (PROC MIXED), which causes of variation contemplated nutritional plan, genotype and interaction as fixed effects and block as random effects. The nutritional plan effect was evaluated by using polynomial regression, separating the linear deviation from linearity effects. The genotype effect was evaluated using the Tukey test at 0.05 significance.

3.3 RESULTS AND DISCUSSION

In the table 2 were presented consumption results. The dry matter intake was influenced ($p<0.05$) by intake level and genotype. When expressed in $BW^{0.75}$, the dry matter intake was lower ($p<0.05$) to Gyr heifers when compared to F1 Holstein-Gyr group, besides Holstein heifers did not differ from the others. Metabolizable energy intake was also lower to Gyr heifers when compared to Holstein and F1 Holstein-Gyr genotypes. Regarding nutritional plan, MEI (Mcal/d) and DMI expressed in kg/d and g/kg of $BW^{0.75}$ was linearly increased with nutritional plan. Body weigh was affect by genotype once Gyr heifers also had less BW in comparison F1 Holstein-Gyr. No effect of interaction was observed for these variables.

Table 2 - Intake from dairy heifers fed different nutritional plans

Variable	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
DMI (kg/d)	4.57^{ab}	3.88^{b}	4.98^{a}	3.19	4.34	5.60	4.53	0.29	0.0071	0.0001_L	NS
DMI (g/$BW^{0.75}$)	64.77^{ab}	63.75^{b}	65.62^{a}	46.00	66.15	77.56	64.80	2.93	0.0198	0.0001_L	NS
MEI (Mcal/d)	11.58^{a}	9.41^{b}	13.60^{a}	8.53	10.14	15.24	11.72	0.95	0.0057	0.0003_L	NS
BW (kg)	279.5^{ab}	240.2^{b}	320.5^{a}	285.4	265.7	298.1	283.5	13.09	0.0081	NS	NS

[1]SEM: standard mean error; [2]N*G: Interaction between nutritional plan and genotype.
DMI: dry matter intake (kg/d); DMI (g/$BW^{0.75}$): dry matter intake (g) per unity of metabolic body weight; MEI: metabolizable energy intake (Mcal/d)
Means with different letters within a row differ significantly according Tukey test, $p<0.05$

In ruminants, physiologic factors, physics and psychogenic seem to control the intake and some difference in this intake capacity has genetic influence, however the magnitude of genetic influence on consumption is difficult to determine (MERTENS, 1994; WESTON, 1982). The *frame* term is referred to size (small, medium and large), animals with less *frame* are lighter and tend to have lower intake than high *frame* animals. Owens (1993) also reported that less *frame* animals may have decrease on intake due the earlier fat accumulation. Another suggest could be related to the digestive tract size of zebu animals, which is morphologically smaller. During the experiment, it was observed in the morning that these Gyr heifers presented some left over in the bins, fact did not found in Holstein and F1 Holstein-Gyr. DMI (g/kg of $BW^{0.75}$) and MEI (Mcal/d) had the same response, 2.2 and 33.8 % lower to Gyr genotype when compared to the others. When looking at intake level, it had a linear response, increasing according the intake. BW was higher ($p<0.05$) to F1 Holstein-Gyr, intermediary to Holstein and less to Gyr. Previous studies have shown the genetic effect on dry matter intake (BORGES, 2000; RENNÓ et al. 2005), which influence body weight and performance and these results are compatible to the results found in the present study.

Daily heart rates were higher ($p<0.05$) to 2.0x maintenance level compared to 1.0x and 1.5x treatments (93.22, 79.37 and 70.50, respectively). Paddock (2010) found that Brangus heifers with low residual feed intake had lower daily heart rate than heifers with high residual feed intake. In the present study, F1 Holstein-Gyr heifers had less ($p=0.05$) daily heart (Table 3) rate when compared to Holstein and Gyr heifers, indicating that F1 crossbred may be more efficient than these others genotypes. The O_2 pulse (mL/min) and heat production (kcal of $BW^{0.75}$) were lower ($p<0.05$) to Gyr heifers compared to Holstein and F1 Holstein-Gyr. Heat production may be influenced by intake level, time spent eating and digesting, production level and environmental conditions (NRC, 1981). Probably Gyr heifers had this inferior heat production (163.2 kcal of $BW^{0.75}$) due the less DMI observed that could be related to any behavioural characteristic in this genotype.

Table 3 - Energy partition in dairy heifers fed different nutritional plans

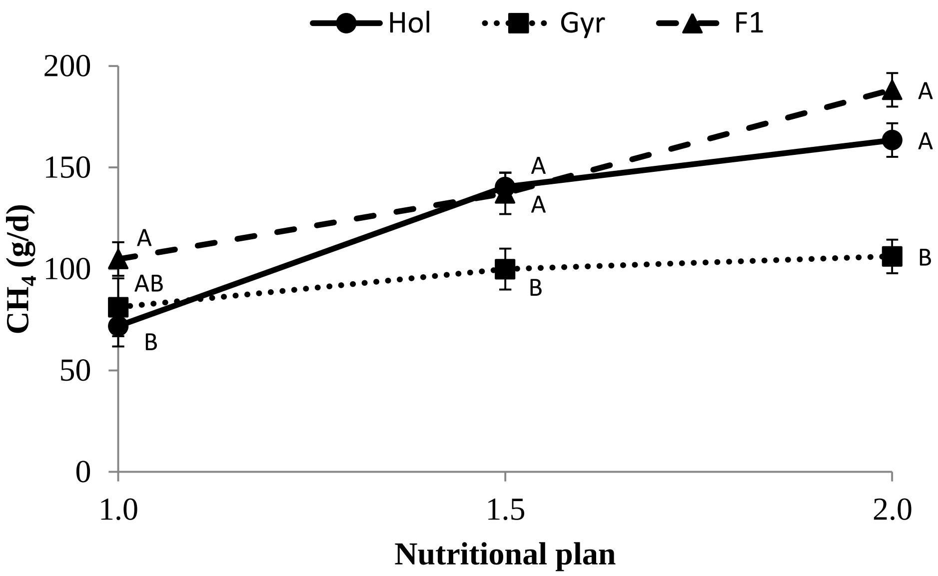
Variable	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
DHR	85.77[a]	86.16[a]	76.00[b]	70.50	79.37	93.22	82.47	2.63	0.0500	0.0002_L	NS
O_2 pulse mL/min	23.03[a]	16.26[b]	25.51[a]	22.80	22.82	21.05	22.13	1.03	0.0043	NS	NS
HP kcal/$BW^{0.75}$	201.0[a]	163.2[b]	181.3[ab]	162.7	193.6	190.4	184.3	5.90	0.0286	NS	NS
CH_4 g/d	132.9[a]	99.9[b]	144.1[a]	89.9	129.5	152.7	128.2	7.88	0.0002	$<.0001_L$	0.0250
CH_4 g/kg DMI	29.55	26.76	29.26	28.31	30.44	27.47	28.72	1.03	NS	NS	NS
CH_4 g/$BW^{0.75}$	1.95[a]	1.67[b]	1.92[a]	1.30	2.00	2.12	1.87	0.09	0.0500	$<.0001_L$	0.0337

[1]SEM: standard of error mean ; [2]N*G: Interaction between nutritional plan and genotype
DHR: daily heart rate in beats per minute; O_2: oxygen pulse; HP: heat production; CH_4 g/ kg of $BW^{0.75}$: CH_4 (g) per kg of metabolic body weight;
Means with different letters within a row differ significantly according Tukey test, p<0.05

There is intake level and genotype interaction ($p<0.05$) for CH_4 emissions in g/animal/d (Figure 1). We observed that in the 1.0x maintenance level F1 Holstein-Gyr presented superior ($p<0.05$) emission of 8.4% and 44.2% if compared to Holstein and Gyr heifers, respectively. In the 1.5x and 2.0x maintenance level Holstein and F1 Holstein-Gyr had similar emissions ($p>0.05$) and Gyr presented 39% and 76% less ($p<0.05$), compared to Holstein and F1 Holstein-Gyr respectively. Oss et al. (2016) comparing different methodologies to measure CH_4 production, including face mask, reported that crossbred bulls (Holstein-Gyr) fed at *ad Libitum* intake had similar CH_4 emission to the present study (152.5 *vs* 152.7 g/animal/d), respectively. This is consistent with previous findings reported in literature where growing dairy Holstein-Friesian x Jersey heifers with similar body weight (261±11 kg) fed *ad libitum* fresh pasture emitted 145 g of CH_4/d (JONKER et al., 2016 no prelo)[2]. The IPCC Tier 1 approach estimates emissions to dairy animals values range from 100 to 323 g of CH_4/d, demonstrating agreement to our results obtained using this alternative methodology. When presented in CH_4 yield (CH_4 in g/kg DMI) it was not find differences ($p>0.05$) among genotypes and nutritional plan, indicating that CH_4 production is related to diet composition and quality (Table 3).

[2] JONKER, A.; MOLANO, G.; KOOLAARD, J.; MUETZEL, S. Methane emissions from lactating and non-lactating dairy cows and growing cattle fed fresh pasture. **Animal Production Science**, 2016. No prelo.

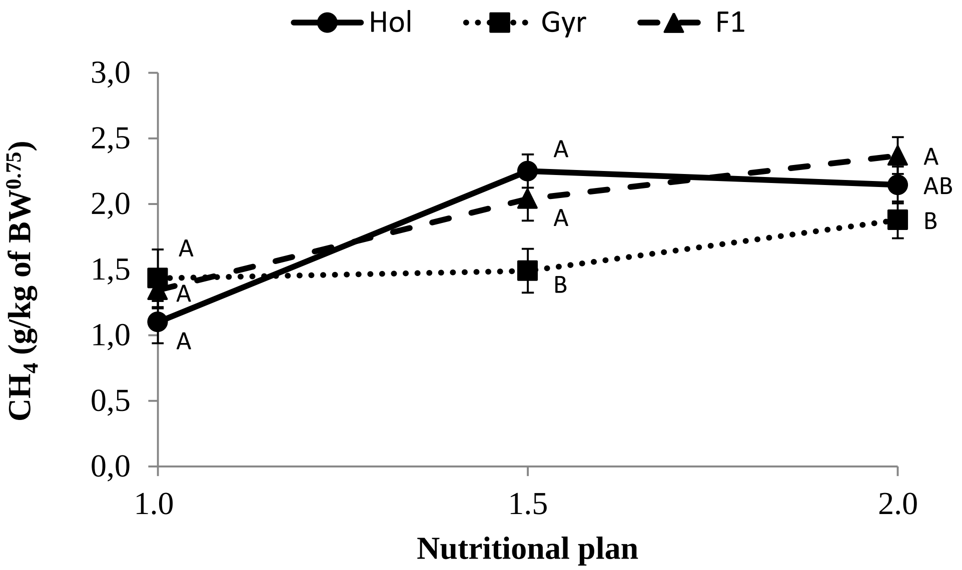
Figure 1 - Effect of interaction between genotype and nutritional plan to CH_4 emission (g/d)



Differences in heifers BW resulted in an interaction ($p<0.05$) among genotype and NP on CH_4 emission (g/kg of $BW^{0.75}$).

In the 1.5x maintenance level Gyr animals ($p<0.05$) showed less emission than Holstein and F1 Holstein-Gyr heifers. When evaluating the 2.0x maintenance level, Gyr genotype emitted a bit higher CH_4 amount (25.5%) but still lowest ($p<0.05$) compared to F1 Holstein-Gyr. Holstein heifers had similar ($p>0.05$) emission, did not differing among genotypes. Our findings suggest that differences on BW were associated with a relative minor difference in age between genotype (Figure 2). Hammond et al. (2015) reported no differences in CH_4 yields (g/kg of $BW^{0.75}$) from lighter (362 kg) or heavier (451 kg) heifers (2.34 *vs*. 2.10, respectively). These authors concluded that BW was confounded with experiment and associated differences on diets and measurements period.

Figure 2 - Effect of interaction between genotype and nutritional plan to CH_4 emission (g/kg of $BW^{0.75}$)



3.4 CONCLUSIONS

The statistical analysis demonstrated reduced DMI, heat production and consequently CH_4 emission (g/d) for Gyr heifers. The results also indicated interaction among genotype and nutritional plan to CH_4 yield (g/kg of $BW^{0.75}$), whereas again lower to Gyr genotype. However, these reduced feed pattern demonstrated from Gyr animals in the present study affected the CH_4 emission in a consistent manner. Future measurements should focus on patterns of CH_4 emission from dairy heifers under different nutritional plans.

As the result of lower genetic merit of Gyr genotype heifers for ADG, these animals have lower DMI even when this data is presented at metabolic weigh basis. As the result of lower DMI (at metabolic body weight basis), those animals also produce less CH_4/kg of $BW^{0.75}$, but not less CH_4/kg DMI. Using DMI as the basis to express CH_4 production, emissions did not differ among different genotypes.

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4 NUTRITIONAL ENERGY REQUIREMENTS AND METHANE EMISSIONS FROM HOLSTEIN, GYR AND F1 HOLSTEIN-GYR GROWING DAIRY HEIFERS

ABSTRACT

The aim of this study was to determinate energy nutritional requirements, energetic partition and methane emissions from growing dairy heifers fed at three different nutritional plans. Thirty-six heifers, 12 Holstein, 12 Gyr and 12 F1 Holstein-Gyr, 17 months old, initial body weight of 295.0±76.8 kg, 172.4±71.9 kg and 317.2±76.5 kg to Holstein, Gyr and F1 Holstein-Gyr, respectively, were distributed into 2 blocks in a 3x3 factorial arrangement (3 nutritional plans x 3 genotypes). Within each latin square, heifers were randomly distributed in 3 different nutritional plans (NP) predicted by NRC (2001), which were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance (lower, intermediary and higher intake, respectively). Heifers were fed a diet consisting of 85.0% corn silage and 15.0% concentrate on dry matter (DM) basis. Measurements were evaluated in respiration chamber (RC) and each heifer spent one 24 hours period in open-circuit chamber. Methane emission methane in grams per day (g/d) was influenced by nutritional plan and genotype. Holstein and F1 Holstein-Gyr genotypes presented 73.4% superior emissions when compared to Gyr heifers, but only when expressed in g/d. Net energy requirements for maintenance (NE_m/kcal $BW^{0.75}$) was 103.9 to Holstein, 79.8 to Gyr and 103.8 to F1 Holstein-Gyr. Metabolizable energy requirements for maintenance (ME_m/kcal $BW^{0.75}$) was 132.6 to Holstein, 116.0 to Gyr and 138.2 to F1 Holstein-Gyr. Differences among Holstein and F1 heifers on NE_m and ME_m were not found, a combined equation for both was formulated, where the net and metabolizable energy requirements for maintenance were 105.2 and 135.0 kcal/$BW^{0.75}$, respectively. It was concluded that those results about nutritional requirements had similarity with available literature from respirometric chambers.

4.1 INTRODUCTION

Indirect calorimetry such as respirometric chamber to measure enteric methane (CH_4) emission from cattle has some advantages including accurate measurements and also heat production data. Therefore, this technique presents some limitations as expense to implantation and maintenance, animal behavior restriction and number of animals to be evaluate simultaneity (JOHNSON and JOHNSON, 1995). Respiration chamber (RC) is also impractical to pastoral simulations preventing interactions within grassland ecosystems (HAMMOND et al., 2015), but is the gold standard methodology to measure CH_4 emission and determine energy partition from ruminants.

An open circuit respiration chamber system have been described by Machado et al. (2016) as a useful tool to estimate nutrients requirements, methane emission and their interaction with climatic conditions. Brazil milk production occupies the 5^{th} position in the international ranking and the cattle formulation diets is still based on nutritional requirements determined in others countries systems, incluinding NRC (1996, 2001; USA), AFRC (1993; United Kingdom), INRA (1988; France) and CSIRO (2007; Australia). Recently, the BR-Corte (VALADARES FILHO et al., 2010) system was an advanced on nutritional requirements from beef cattle. However, about nutritional requirements to dairy cattle only 2 studies published from RC are available on literature and seems a long way to generate dairy cattle database and a future system. Thus, filling this gap could be an efficient alternative on productivity and economicity in Brazilian dairy cattle systems.

Since 2013, the Multi-use Complex on Livestock Bioefficiency and Sustainability of the Brazilian Agricultural Research Corporation (Embrapa Dairy Cattle) has been focused on bioenergetics studies of representative genotypes of Brazilian dairy herd and searching for strategies to mitigate and reduce global livestock greenhouse gases (GHG) emissions. GHG emissions from agriculture and livestock produced in Brazil were accounted in 35.1% of the national GHG inventory (MCTI, 2013). The large cattle population is the major source to this contribution, thus developing countries contribute considerable to global methane emissions.

The objective of this study was to evaluate 3 dairy genotypes groups in tropical conditions fed at different 3 nutritional plans to determine energy nutritional requirements, energetic partition and methane emissions. It was hypothesized that genotype has no influence on methane emission but they have differences on energy nutritional requirements.

4.2 MATERIAL AND METHODS

This study followed the Ethics Committee in Animal Use of the University of Sao Paulo. The trial was conducted at The Multi-use Livestock Complex of Bioefficiency and Sustainability at Embrapa Dairy Cattle, Coronel Pacheco, MG, Brazil from June to August, 2013.

Animals were allocated into two blocks in a factorial arrangement 3x3 (3 genotypes and 3 nutritional plans). Thirty six dairy heifers, 12 Holstein, 12 Gyr and 12 F1 Holstein x Gyr were randomly subdivided in different nutritional plans (NP) predicted by NRC (2001), which were (1) 1.0x maintenance; (2) 1.5x maintenance and (3) 2.0x maintenance. Throughout the present study, heifers diets were total mixed ration (*TMR*) of corn silage combined with concentrates and the proportion between silage and concentrate was 85:15 based on the dry matter (DM) and the chemical diet composition is described in Table 1. The *TMR* from 1.0x and 1.5x maintenance levels had the same composition, differing just in the amount offered to the heifers. Concentrate from 1.0x and 1.5x maintenance levels were composed of soybean meal (81.4%), urea (7.1%), mineral mix (4.8%), ammonium sulfate (3.5%) and mineral salt (3.2%). The 2.0x maintenance level concentrate was composed of soybean meal (79.1%), ground corn (9.4%), urea (4.4%), mineral mix (2.9%), ammonium sulfate (2.2%) and mineral salt (2.0%). The feed was provided once daily in the morning (08:00 h) while the chambers doors were open and the measurements started. All feeds offered were weighed daily and samples dried to determine DM content; dry matter intake (DMI) was calculated per animal daily.

Table 1 - Formulation and chemical composition of experimental diet

Item	Nutritional plan	
	1.0 and 1.5x	2.0x
Formulation	g/kg	
Corn silage	851.0	806.0
Soybean meal	120.0	153.0
Ground corn	0.0	18.0
Urea	11.0	9.0
Ammonium sulfate	5.0	4.0
Mineral mix[a]	8.0	6.0
Mineral salt	5.0	4.0
Total	1000	1000
Composition	g/kg DM	
Dry matter	356.7	356.6
Crude protein	166.1	150.4
Ether extract	38.1	38.4
NDF[b]	472.6	473.3
NFC[c]	254.1	268.5
Energy density	Mcal/kg	
GE[d]	4.43	4.46
ME[e]	2.62	2.73

[a]Guarante levels: Ca:190 g/kg; P:60 g/kg; Na:70 g/kg; Mg: 20 g/kg; Co:15 mg/kg; Cu:700 mg/kg; Mn:1.600 mg/kg; Se:19 mg/kg; Zn:2.500 mg/kg e I:40 mg/kg.
[b]Neutral detergent fiber;
[c]Non-fibrous carbohydrates;
[d]gross energy;
[e]Metabolizable energy determined in metabolism trial

Heifers were initially held in metabolism stall before RC measurements and nutrients digestibility measured (faeces and urine). Body weight was measured daily when the heifers entered and left the respiration chambers (RC) and the average was 295.0±76.8 kg to Holstein, 172.4±71.9 kg to Gyr and 317.2±76.5 kg to F1 Holstein-Gyr. The heifers had been previously accustomed to the respirometry procedures and equipment before CH_4 measurements.

Urinary creatinine concentration was used as an indicator of urine output because total urine output was not obtained in this trial. Urine samples of 50 mL were collected from all animals at four times per day (07:00, 13:00, 19:00 and 01:00 h) by massage on the vulva, filtered in by triple layer gauze, elaborating composite samples of 200 mL per animal. Samples were stored at -15ºC for later analysis of creatinine by automatic biochemical analyzer. Urine volume was estimated using creatinine concentration as marker and assuming a daily creatinine excretion of 32.27 mg/d (CHIZZOTTI et al., 2004).

The open-circuit respirometry system used to methane emission measurements consists of two RC with a total volume of 21.1 m^3. The room temperature and relative humidity were kept at 23 ± 0.5 °C and 55 ± 5%, respectively. Each chamber was equipped with it own air treatment unit with a recirculating fan (800 m_3/h) and air filters. Each chamber was fitted with an air outlet with a filter box (CSL-851-200HC, Solberg Manufacturing Inc., Itasca, USA) with the air being continuously drawn into the chamber by a sealed rotary pump connected to a mass flow regulator (FlowKit model FK-500, Sable Systems International, Las Vegas, NV, USA). The two chambers shared a common gas analysis and data acquisition system (Sable Systems International, Las Vegas, USA). Gas samples from the two chambers and the ambient air (baseline) were continuously sampled at 0.5 L/min. A diaphragm sub-sampling pump (SS-4 Sub-Sampler Pump) was used to deliver the sub-samples of air to the CH_4 analyser at 200 mL/min. Every 15 min, a subsample was taken over 5 min from ambient baseline air and from each chamber. The samples were delivered to the respirometry system (Sable Systems International, Las Vegas, NV, USA), first for analysis of water vapor (RH-300 Water Vapor Analyzer) and then for gases analysis, O_2, CO_2 and CH_4 (MA-10, Sable Systems, Henderson, NV). The CH_4 analyzer (zero and span) was calibrated as described above. Recovery of CH_4 in each chamber was estimated using a portable mass flowmeter with a totalizer function (MC-50SLPM-D, Alicat Scientific Inc., Tucson, AZ, USA), with recoveries estimated at 98.0%. Data acquisition and analysis software (ExpeData v.1.7.5, Sable Systems International, Las Vegas, USA) was used to estimate O_2, CO_2 and CH_4 concentrations, flow rate, temperature, barometric pressure and water vapour pressure during the measurement period. Gas measurements were corrected for differences in water vapor, lag time and drift, with CH_4 emission (L/min) being calculated for each chamber at 15 min intervals.

There were 2 respiration chambers and the heifers were pared beside each other to enabled contact visual by windows. Each heifer spent one 24 hours period in an open-circuit RC for gas exchange measurements and heat production (HP) in fed animals. After 24h, measurement was interrupted and heifers removed for cleaning chambers. Respiratory quotient was calculated as the reason between carbon dioxide production (CO_2) and oxygen consumption (O_2). HP was determined for the continuous measurement of oxygen consumption, carbon dioxide production and CH_4 emissions, according Brower (1965) equation. Methane emission was calculated by cumulative production over the measurement period, adjusted for 24 hour period and corrected by the recovery factor of each chamber. Energy partition was calculated by subtracting the gross energy consumed by the energy losses from faeces, urine, methane and daily heat production. Digestible energy (DE) and metabolizable energy (ME) diet concentrations (Mcal/kg) were obtained as the reason between energy intake and dry matter intake in digestibility nutrients measurements. The diet metabolizability (q) was calculated by the ratio between ME and gross energy ingested, as described by AFRC (1993). Net energy requirement for maintenance (NE_m) was calculated as the antilogarithm of intercept regression of the logarithm of HP due metabolizable energy intake (MEI). By the iterative method, the point where MEI equals to HP can be determined, and this point was considered the metabolizable energy requirement for maintenance (ME_m). The efficiency of use of metabolizable energy for maintenance (*km*) was obtained from the relation between the NE_m and the ME_m (LOFGREEN and GARRET, 1968).

4.2.1 Statistical analysis

Data were analysed by Statistical Analysis System, being previously verified the normality of residuals by the Shapiro-Wilk test and the homogeneity of variances compared by the Levene test. These data were submitted to the analysis of variance, using the mixed models procedure (PROC MIXED), which causes of variation contemplated nutritional plan, genotype and interaction as fixed effects and block as random effects. The nutritional plan effect was evaluated by using polynomial regression, separating linear and deviation from linearity effects. The genotype effect was evaluated using the Tukey test at 0.05 significance.

4.3 RESULTS AND DISCUSSION

The dry matter intake kg/d (Table 2) was lower in Gyr breed ($p<0.05$) compared to Holstein and F1 Holstein-Gyr and the same pattern was observed to DMI (g/kg of $BW^{0.75}$). In both, a linear ($p<0.05$) increase on DMI was observed. Silva (2011) in a first nutritional requirements study (respirometric chamber) from dairy animals found similar DMI to Holstein heifers, 4.41 *vs* 4.67 kg/d and 61.80 *vs.* 65.73 g/kg of $BW^{0.75}$, compared to current study. Evaluating the DMI from Gyr and F1 Holstein-Gyr heifers fed Tifton-85 hay (*Cynodon spp*.), Lage (2011) found values of 66.5 and 75.8 g/kg of $BW^{0.75}$, respectively. These values are superior that current study but these differences may be related due the body weight, diet composition and form of conservation. In this context, is imperative the development of national research to obtain database to formulate equations for prediction of dry matter intake of dairy heifers, considering main crossing strategies

Table 2 - Dry matter intake from dairy heifers fed different nutritional plans

Variable	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
DMI (kg)	4.67[a]	2.92[b]	5.27[a]	2.82	4.75	5.30	4.28	0.40	0.0030	0.0020_L	NS
DMI (g/$BW^{0.75}$)	65.73[ab]	60.66[b]	69.38[a]	44.63	69.31	81.83	65.26	4.00	0.0456	0.0001_L	NS

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype
DMI: dry matter intake (kg/d); DMI (g/$BW^{0.75}$): dry matter intake in grams of kg of metabolic body weight;
Means with different letters within a row differ significantly according Tukey test, $p<0.05$

Oxygen consumption and carbon dioxide production (L/kg of $BW^{0.75}$) were 19.3% and 21.8% lower ($p<0.05$) respectively to Gyr heifers compared to the others and both increased linearly ($p<0.05$) due nutritional plan. Respiratory quotient (RQ) did not differ ($p>0.05$) between genotypes and it was found linear effect ($p<0.05$) to nutritional plan. RQ ranged from a minimum value of 1.06 to 1.15 (Table 3).

Chwalibog (2004) reported that oxygen consumption, carbon dioxide and methane production associated to urinary nitrogen excretion can be used to calculated the heat production, which were higher ($p<0.05$) to F1 Holstein-Gyr in comparison to Gyr, but Holstein heifers did not differing among genotypes. HP also had a linear effect where animals from 2.0x maintenance level ($p<0.05$) showed 46.9% higher HP in comparison to 1.0x maintenance level.

Ferreira (2014) evaluating F1 Holstein-Gyr crossbred bulls under different dietary conditions found that increasing on intake level had effect ($p<0.05$) on gas exchange. Bulls fed at *ad libitum* showed greatest O_2 consumption and CO_2 production when compared to maintenance treatment. The nutritional plan also has effect on animal metabolism, once Ferrel and Koong (1987) demonstrated that animals fed on maintenance level had reduced O_2 consumption from liver and kidneys.

Table 3 - Gas exchange, respiratory quotient and heat production from growing dairy heifers fed different nutritional plans

Item	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
O_2	27.03^{ab}	24.21^{b}	30.72^{a}	21.15	30.28	30.54	27.32	1.38	0.0159	0.0008$_L$	NS
CO_2	30.75^{a}	26.87^{b}	34.73^{a}	22.71	34.35	35.29	30.78	1.70	0.0108	0.0003$_L$	NS
RQ	1.13	1.09	1.12	1.06	1.13	1.15	1.19	0.01	NS	0.0038$_L$	NS
HP	140.36^{ab}	124.94^{b}	159.23^{a}	108.32	157.09	159.13	141.51	7.29	0.0136	0.0006$_L$	NS

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype
O_2: oxygen gas (L/kg of $BW^{0.75}$); CO_2: carbon dioxide (L/kg of $BW^{0.75}$); RQ: CO_2 production/O_2 consumption; HP: heat production kcal/kg of $BW^{0.75}$;
Means with different letters within a row differ significantly according Tukey test, $p<0.05$.

Methane production (Table 4) was affect ($p<0.05$) by genotype and nutritional plan only when expressed in g/d. The CH_4 production (g/d) was higher to Holstein and F1 Holstein-Gyr (117.47 g/d) genotypes presenting 73.4% superior emissions when compared to Gyr (67.73 g/d) heifers. As described anteriorly on chapters 1 and 2, Gyr heifers presented lower dry matter intake, resulting in less CH_4 emissions. Overall, the hypothesis that CH_4 emission in g/d would be increased by intake level was supported in the present study. Many authors reported that diet composition, intake and production level has effect on CH_4 emissions. Pancoti (2015) also evaluating CH_4 emissions by respirometric technique found similar results observed in current study, where Holstein and F1 Holstein-Gyr dairy heifers presented 82% higher emission than Gyr, due the greatest DMI and nutrients ingested. Weiske et al. (2006) have reported the importance of interaction between different dairy cows genotypes and feeding levels on GHG emissions. In a long study from Holstein-Friesian dairy cows, Chagunda et al. (2008) observed the effect of genotype and feeding regime (low or high forage system) on enteric CH_4 emissions per kg of milk estimated by equation described by Yates et al. (2000) and found the high genetic merit cows on a low forage diet had the lowest enteric CH_4 emissions per kg of milk while the control genetic merit on high forage diet had the highest enteric CH_4 emissions per kg of milk, 12.2 *vs.* 18.5 g/kg of milk. Some authors (NKRUMAH et al. 2006; HEGARTY et al. 2007) investigating feedlot cattle selected for residual feed intake (RFI) reported that low RFI animals (feed-efficient) demonstrated lower CH_4 yields compared to high RFI (feed-inefficient animals).

Australia and New Zealand have focused on the development of new technologies that can contribute to reduce overall emissions from agriculture, investigating natural variation on CH_4 emissions from sheep, screening low and high CH_4 yielding animals with the long term goal of selecting animals with lower CH_4 yields without compromising their productivity or reproductive ability (SHI et al., 2014).

Generally, an increase in production efficiency in terms of final product is associated with a decrease in enteric methane emissions per liter of milk, beef or gain. The development and adoption of new technologies potentially sustainable are crucial to mitigate GHG, given the increasing global population and associated demand for food. In this study, it was not found any effect on CH_4 emissions by dry matter, organic matter and neutral detergent fiber ingested or digested, which means that genotype has no influence on CH_4 production.

Gross energy intake (GEI) and faecal gross energy (Mcal/d) had interaction ($p<0.05$) among treatments, increasing linearly due nutritional plan (Table 5). Faecal gross energy when evaluated by %GEI was found nutritional plan effect ($p<0.05$) and the 1.5x maintenance nutritional plan had 10.5 and 6.5% superior losses than 1.0x and 2.0x maintenance nutritional plan, respectively. The digestible energy intake (Mcal/d) reflects the faecal energy losses and had the same pattern observed to the GEI, e.g. interaction ($p<0.05$) among treatments.

Table 4 - Methane emission from dairy heifers fed different nutritional plans

Item	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
g/d	105.01[a]	67.73[b]	129.93[a]	67.09	114.93	116.63	99.55	9.41	0.0007	0.0022_L	NS
g/kg DM_{int}	22.83	20.87	25.13	22.62	24.25	21.96	22.94	0.89	NS	NS	NS
g/kg OM_{int}	24.53	22.47	27.00	24.85	26.09	23.64	24.67	0.95	NS	NS	NS
g/kg NDF_{ing}	50.08	44.90	53.75	48.10	51.45	49.18	49.58	1.78	NS	NS	NS
g/kg DM_{dig}	32.39	29.05	35.32	31.44	34.60	30.73	32.26	1.27	NS	NS	NS
g/kg OM_{dig}	34.20	30.61	37.21	33.05	36.49	32.48	34.01	1.36	NS	NS	NS
g/kg NDF_{dig}	88.89	75.55	92.16	79.15	89.84	87.60	32.26	1.27	NS	NS	NS

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype
Means with different letters within a row differ significantly according Tukey test, p<0.05

Urinary gross energy losses showed linear decrease ($p<0.05$) with average of 4.77% of GEI. Ferreira (2014) in a bioenergetic study evaluating crossbred F1 Holstein-Gyr bulls in 3 different intake levels found similar values (4.54% of GEI) observed in current trial. These data supported findings reported by Van Soest (1994), where urinary losses represent 3-5% of gross energy intake. Methane losses (%GEI) differed among genotypes and F1 Holstein-Gyr ($p<0.05$) had the greatest loss. When presented in nutritional plan, it was detected linear increase ($p<0.05$). The higher nutritional plan demonstrated 5.79% of CH_4 losses from GEI. Published data from Johnson and Johnson reports values of CH_4 losses equal to 6% GEI. Our results of energetic losses from urine (4.77%) and methane (5.79%) are in concordance with literature consulted.

Metabolizable energy intake (MEI) also presented interaction ($p<0.05$) among treatments, increasing linearly due digestible energy intake. The ARC (1980) reports that the relation between metabolizable energy and digestible energy is approximately 0.82 and AFRC (1993) suggest values ranging from 0.80 to 0.86. These values are in concordance that was found in current study, ME/DE=0.84 on average.

An interaction among genotype and nutritional plan was found to heat production (Mcal/d). Gyr heifers showed reduced HP in 63.7%, 18.1% and 76.4% compared to Holstein and F1 Holstein-Gyr in the lower, intermediary and higher nutritional plan, respectively, probably due the less body weight from these heifers and consequently reduced DMI (Figure 1).

Figure 1 - Effect of interaction between genotype and nutritional plan to HP (Mcal/d)

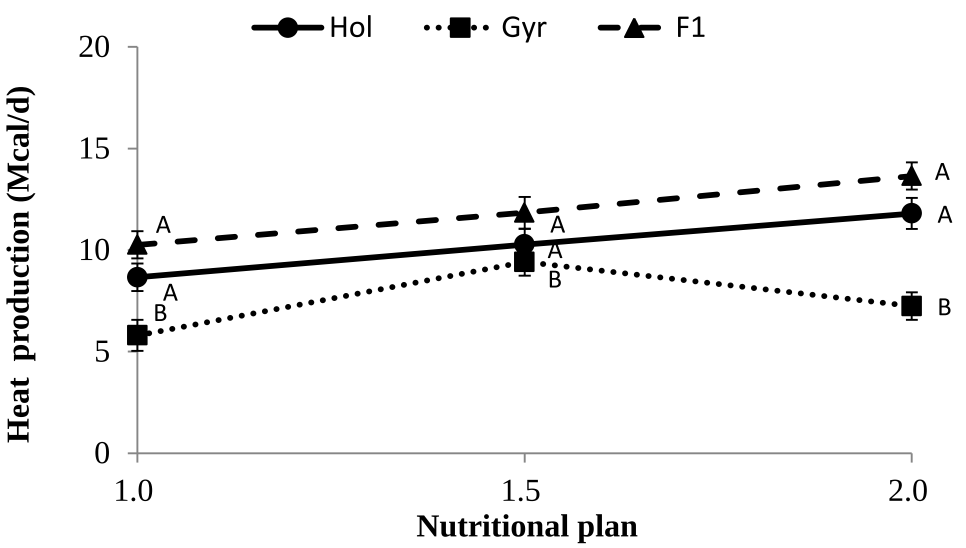


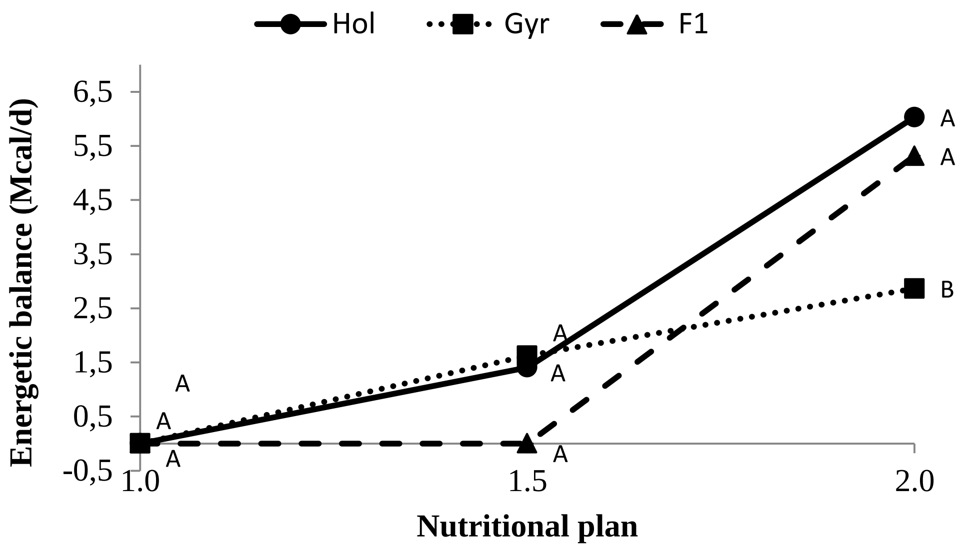
Table 5 - Energetic balance from growing dairy heifers fed different nutritional plans

Item	Genotype			Nutritional plan			Mean	[1]SEM	Probability		
	Hol.	Gyr	F1	1.0x	1.5x	2.0x			Genotype	NP	[2]N*G
GEI (Mcal/d)	20.38^{a}	15.37^{b}	23.16^{a}	13.68	19.97	25.22	19.61	1.24	<0.001	$<0.001_{L}$	0.0048
GE faecal (Mcal/d)	5.95^{a}	4.31^{b}	6.64^{a}	3.74	6.00	7.17	5.63	0.37	<0.001	$<0.001_{L}$	0.0021
GE faecal (%GEI)	28.92	28.06	28.70	27.30	30.16	28.32	28.55	0.39	NS	0.0104_{D}	NS
DEI (Mcal/d)	14.43^{a}	11.05^{b}	16.52^{a}	9.94	13.96	18.05	13.99	0.88	<0.001	$<0.001_{L}$	0.0036
GE urine (Mcal/d)	0.84	0.76	1.03	0.81	0.84	0.97	0.88	0.05	NS	NS	NS
GE urine (%GEI)	4.32	5.20	4.70	6.05	4.19	3.97	4.77	0.30	NS	0.0033_{L}	NS
GE CH_4 (Mcal/d)	1.37^{b}	0.98^{c}	1.68^{a}	0.97	1.43	1.63	1.34	0.08	<0.001	$<0.001_{L}$	0.0181
GE CH_4 (%GEI)	4.73^{b}	3.49^{c}	5.90^{a}	3.56	4.77	5.79	4.70	0.30	<0.001	$<0.001_{L}$	NS
MEI (Mcal/d)	12.22^{a}	9.31^{b}	13.81^{a}	8.15	11.70	15.48	11.76	0.77	<0.001	$<0.001_{L}$	0.0097
q (EM/ED)	0.59	0.60	0.59	0.60	0.58	0.61	0.59	0.004	NS	NS	NS
ME/DE	0.84	0.84	0.83	0.82	0.84	0.85	0.84	0.004	NS	0.0047_{L}	NS
HP (Mcal/d)	10.1^{b}	7.65^{c}	11.87^{a}	8.51	10.32	10.83	9.87	0.45	<0.001	0.0001_{L}	0.0452
HP (%CEB)	53.6	50.68	53.47	62.12	51.93	43.14	52.41	1.52	NS	$<0.001_{L}$	NS
EB (Mcal/d)	2.10	1.65	1.94	-0.35	1.36	4.62	1.89	0.43	NS	0.0001_{L}	0.0111

[1]SEM: standard error of mean; [2]N*G: Interaction between nutritional plan and genotype;
GEI: gross energy intake (Mcal/dia); GE fecal: faecal gross energy (Mcal/d); DEI: digestible energy intake;
GE CH_4: methane gross energy losses (Mcal/d);
HP:daily heat production; EB: energetic balance; q: diet metabolizability;
Means with different letters within a row differ significantly according Tukey test, $p<0.05$

Energetic balance demonstrates interaction ($p<0.05$) among treatments. In the 1.0x and 1.5x maintenance nutritional plan, we found no differences between genotypes. But, looking at 2.0x nutritional plan, Gyr heifers presents inferior ($p<0.05$) EB in comparison to Holstein and F1 Holstein-Gyr, that did not differ between each other ($p>0.05$). The EB was calculated by the difference between MEI and HP plus others, so the higher nutritional plan resulted in greatest EB, reflecting larger amount of ME available above of maintenance requirements.

Figure 2 - Effect of interaction between genotype and nutritional plan to EB (Mcal/d)



In our study the NE_m (kcal/$BW^{0.75}$) found was 103.9 to Holstein, 79.8 to Gyr and 103.8 to F1 Holstein-Gyr. Differences among Holstein and F1 Holstein-Gyr heifers on NE_m and ME_m were not found, so it was formulated a combined equation for both, where the net and metabolizable energy requirements for maintenance were 105.2 and 135.0 kcal/$BW^{0.75}$, respectively (Table 6).

Informations about energy requirements of dairy cattle are sparse on tropical conditions, especially South America. As described in NRC (1996), the environment had important effects on maintenance requirements of cattle. The NE_m requirement reported by the NRC (2001) for dairy heifers is 80 kcal/$BW^{0.75}$ and intermediary to crossbred animals. Data found in present study showed that crossbreed F1 Holstein-Gyr heifers had similar (103.8 *vs* 103.9 kcal/$BW^{0.75}$) maintenance energy requirements when compared to Holstein, showing that this crossbreeding is as exigent as pure genotypes. However, our results about NE_m requirements from Holstein and F1 Holstein-Gyr heifers are superior than those described by NRC (2001) and similar to Gyr heifers. Previously reported, Lofgreen and Garrett (1968) found NE_m requirements of 77 kcal/ $BW^{0.75}$, close to NE_m obtained to Gyr heifers, which was 79.8 kcal/$BW^{0.75}$. Ferrell et al. (1976) suggests that higher internal organ size in *Bos taurus taurus* than *Bos taurus indicus* could explain this difference on NE_m requirements among genotypes. The NRC (2000) also published that zebu breeds required 10% less of energy requirements than taurine breeds. Reduced maintenance energy in *Bos taurus indicus* is due to the lower production genetic potential, characteristic developed by adaptation to the environment conditions, less favourable (CSIRO, 2007; NRC 1996). Chizzotti et al. (2008) in a meta-analysis compiled from 389 Nellore purebred bulls and their taurine crossbred estimated the net energy requirements by comparative slaughter, where the HP is not directly measured, but obtained by the difference between metabolizable energy intake and retained energy in the empty body. This author reported 67 kcal/$BW^{0.75}$ of net energy maintenance requirement.

The ME_m (kcal/$BW^{0.75}$) observed in the current study was 132.6 to Holstein, 116.0 to Gyr, and 138.2 to F1 Holstein-Gyr. In a meta-analysis from 32 respirometric chambers experiments involving different dairy cows genotypes (Holstein-Friesian, F1 Jersey-Holstein Friesian, Norwegian and Norwegian-Holstein Friesian), Dong et al. (2015) demonstrated no difference on ME_m requirements, 164.4 and 163.9 kcal/$BW^{0.75}$ to Holstein-Friesian crossbred and Norwegian cows. Indicating that crossbreeding Holstein-Friesian cows may have a little effect on the basal metabolic rate of Holstein-Friesian dairy cows. Solis et al. (1998) evaluating energy requirements from beef and dairy cattle reported that animals with beef adaptability presented 27% less ME_m requirements than dairy cattle. This author found 115.7 and 140.4 ME_m (kcal/$BW^{0.75}$) requirements from Holstein and Jersey, respectively. National data also from respirometric chambers reported that F1 crossbred Holstein-Gir presents 17.8% higher ME_m requirements than Gyr, finding 146.6 and 120.05 (kcal/$BW^{0.75}$) to F1 Holstein-Gyr and Gyr, respectively (LAGE, 2011). When comparing this previous study (LAGE, 2011) with current data, we found no differences on ME_m requirements.

The efficiency of the use of metabolizable energy for maintenance (*km*) that is obtained from the relation between the NE_m/ME_m detected in this study was superior to those recommend by NRC (2001) and CNCPS (FOX et al., 2004), which is 0.64. CSIRO (2007) suggested many factors that could affect the *km*, including: age, environment, gender and genotype. In this experimental conditions, it was found that Gyr present *km* 11% lower compared to Holstein and F1 Holstein-Gyr. Due the gap on Brazilian published data about nutritional requirements from dairy cattle, it is difficult to make an effective data comparison, but these results had similarity with available literature from respirometric chambers.

Table 6 - Regression parameters of heat production (kcal/BW$^{0.75}$) logarithm due metabolizable energy intake (kcal/BW$^{0.75}$) from growing dairy heifers by respirometric technique

Genotype	Intercept (a)	Coeficient (b) x10000	n	R^2 adj.	RSE	NE_m	ME_m	*km*
Hol.	2.01 ± 0.04	7.84 ± 2.24	12	0.61	0.034	103.9	132.6	0.78
Gyr	1.90 ± 0.02	13.52 ± 1.56	12	0.89	0.019	79.8	116.0	0.69
F1	2.01 ± 0.02	9.39 ± 1.03	12	0.91	0.017	103.8	138.2	0.78
Hol. and F1	2.02 ± 0.01	8.77 ± 0.99	24	0.84	0.020	105.2	135.0	0.78

R^2 adj: coefficient of determination; RSE: residual standard error; NE_m: net energy requirements for maintenance; Me_m: metabolizable energy requirements for maintenance;
km: efficiency of the use of metabolizable energy for maintenance

4.4 CONCLUSIONS

Gyr heifers presented less methane emission, heat production and nutritional energy requirements. Less methane emission is clearly explained due the lower dry matter intake observed in our study, but lesser heat production and nutritional energy requirements cannot be explained by this reason. Holstein and F1 Holstein-Gyr have no differences on net and metabolizable energy maintenance requirements but superior than those described on foreign nutritional systems. Given these reasons, is required an effort on further researches to generate a database to elaborate a nutrient requirement system for dairy genotypes used in tropical conditions.

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5 GENERAL CONCLUSIONS

Our results indicate that Gyr heifers presented higher crude protein digestibility than Holstein and F1-Holstein Gyr. Gyr genotype also demonstrates reduced dry matter and nutrients intake and consequently lower methane emission when expressed in grams per day. Increasing heifers performance by live weigh gain is an important benefit to reduce methane emissions per unit of animal product, which means a strategy to mitigate GHG. Finally, Gyr heifers had lesser net and metabolizable energy requirements compared to Holstein and F1-Holstein-Gyr. However, the present study found that growing Holstein and F1-Holstein-Gyr dairy heifers presented no differences on energy requirements, but superior than those described on foreign nutritional systems.

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