DANIELA BATISTA OSS

ENERGY AND PROTEIN REQUIREMENTS OF CROSSBRED (HOLSTEIN X GYR) YEARLING BULLS AND ASSESSMENT OF TECHNIQUES FOR MEASURING METHANE EMISSIONS AND ENERGY EXPENDITURE OF CATTLE

Thesis submitted to the Federal University of Viçosa as partial fulfillment of the requirements of the Graduate Program in Animal Science to obtain the degree of *Doctor Scientiae.*

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APPROVED: February 25, 2016.

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“And once the storm is over, you won’t remember how you made it through, how you managed to survive. You won’t even be sure, whether the storm is really over. But one thing is certain. When you come out of the storm, you won’t be the same person who walked in. That’s what this storm’s all about.” – Haruki Murakami

DEDICATION

I would like to dedicate this thesis to my father (Gilvan Ribeiro Batista), my mother (Rozânia Maria Oss Batista) and my sister (Bruna Batista Oss), for all of their love and support.

Also, to my grandfather (Adão Ruberval Batista) – the main responsible for all of my passion for animal production.

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BIOGRAPHY

Daniela Batista Oss, daughter of Gilvan Ribeiro Batista and Rozânia Maria Oss Batista, was born in Montanha/ES – Brazil on February 23, 1985.

She started the undergrad in Animal Science at Universidade Federal do Espírito Santo (UFES), and obtained a Bachelor of Science degree in Animal Science in 2009. In 2010, she started the Master Science program, with major in forage and pastures and ruminant nutrition at the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). In February 15, 2012, she obtained a *Scientiae Magister* Degree in Animal Science.

In the same year, she started her doctorate program with a major in ruminant nutrition and dairy cattle production at the Universidade Federal de Viçosa (UFV). From July 2014 to July 2015, she was a visiting student at the Agriculture and Agri-Food Canada (AAFC), in Lethbridge, AB – Canada, where part of her doctorate program was performed.

On February 25th 2016, she submitted her dissertation to the thesis committee to obtain the *Doctor Scientiae* degree in Animal Science.

TABLE OF CONTENTS

ABSTRACT

OSS, Daniela Batista, D.Sc. Universidade Federal de Viçosa, February, 2016. **Energy and protein requirements of crossbred (Holstein x Gyr) yearling bulls and assessment of techniques for measuring methane emissions and energy expenditure of cattle.** Adviser: Marcos Inácio Marcondes. Co-adviser: Luiz Gustavo Ribeiro Pereira.

The facts that justify the development of this work are: 1 – There is few information about nutrient requirements of crossbreeds Holstein x Gyr cattle, which is widely used in Brazil; 2 – Pressure to reduce livestock greenhouse gases has prompted greater interest in research to reduce enteric methane emissions from ruminants and consequently in the development and validation of techniques for measuring methane; 3 – Considering that energy expenditure is one of the most element in overall energy budget of cattle, to have measurement techniques that are suited to different production conditions it is important to studies that seek understand energy metabolism and partition of cattle. Therefore, this work was developed from three studies. The first one aimed to estimate the energy and protein requirements of crossbred (Holstein × Gyr) yearling bulls. Twenty-four 10 months old bulls (initial body weight = 184.1 ± 23.36 kg) were used in a comparative slaughter trial. Six bulls were slaughtered at the beginning of the experiment as the reference group to estimate initial empty body weight (EBW) and energy and protein contents of the remaining animals. The remaining bulls were assigned to a completely randomized design with 3 DM intake levels, with 6 replicates. The levels of DM intake were: 1.2% of BW, 1.8% of BW and *ad libitum* – target orts 5%. Bulls were fed a diet consisting of 59.6% corn silage and 40.4% concentrate on a DM basis. The equation adjusted for the relationship between EBW and BW was $EBW = 0.861_{\pm 0.0031} \times BW$. While the relationship between empty body gain (EBG) and average daily gain (ADG) may be demonstrated using the equation: $EBG = 0.934_{\pm 0.0111} \times ADG$. The net energy requirements for maintenance (NE_m) were $74.8_{\pm 2.89}$ kcal/$EBW^{0.75}$/d, the metabolizable energy requirements for gain (ME_m) were 120.8 kcal/$EBW^{0.75}$/d. The efficiency of the use of metabolizable energy for maintenance ($\boldsymbol{k}_m$) was 61.9%. The equation adjusted to estimate the net energy for gain (NE_g) was: $NE_g = 0.049_{\pm 0.0011} \times EBW^{0.75} \times EBG^{0.729 \pm 0.0532}$. The efficiency of the use of metabolizable energy for gain ($\boldsymbol{k}_g$) observed was 30.8%. The metabolizable protein requirements for maintenance (MP_m) was 3.05 g/kg $BW^{0.75}$. The equation

adjusted to estimate the net protein requirements for gain was: $NP_g = (87.138_{\pm 65.1378} \times EBG) + (40.436_{\pm 21.3640} \times NE_g)$. The efficiency of the use of metabolizable protein for gain (***k***) observed was 53.6%. We conclude that the estimates of energy and protein requirements were not similar to those estimated from the other assessed systems, thus we recommend using the estimates herein presented to balance diets of crossbred (Holstein × Gyr) growing bulls. The second study aimed to compare short- term measurement (30 min/d for 3 d) face mask system (FM), with SF_6 tracer and respiration chamber (RC) techniques for measuring methane emissions. The same animals, treatments and diet used in the first study were used in the second and third study. Methane emissions were measured first using the SF_6 tracer technique, followed by the FM and RC techniques, respectively. Methane collection was initiated for a period of 24 h, with the procedure repeated on 5 consecutive days. Two weeks later, the FM technique was evaluated and a single 30-min CH_4 measurement was performed each day for 3 d by collecting measurements 6 h after feeding. After 4 weeks, methane emissions from bulls were estimated using indirect open-circuit respiration chambers (RC) by placing bulls in the chambers for two 24 h periods of methane measurements. The concordance correlation coefficient (CCC) for CH_4 emission (g/d) were: 0.82, 0.82 and 0.74 for comparisons of SF_6 *vs* RC, FM *vs* RC and FM *vs* SF_6, respectively. Methane emission adjusted for differences in DMI did not differ among techniques, averaging 21.5 g/kg DMI (P=0.238). However, the day-to-day (21.3%) and animal-to-animal (13.4%) variation in CH_4 yield was greater for the FM technique as compared to SF_6 (18.8% and 6.8%) and RC (12.9% and 7.5%) techniques. This higher variation has a negative impact on power of an experiment using FM, and it would require more animals (replicates) to detect treatment difference with 80% of power. The third study aimed to assess the O_2P-HR (oxygen pulse (O_2P) and heart rate (HR)) technique with respiration chamber (RC) and comparative slaughter (CS) methods for measuring energy expenditure (EE) of cattle. The O_2P-HR method is an alternative technique for measuring EE, that is based on long-term measurements (24 h periods) of the HR of free-range animals, and on short-term measurement of oxygen pulse (O_2P; mL of O_2 consumed/heart beat) which is measured by attaching a face mask (FM) to the animal's nose shortly (20 min). For both comparison (O_2P-HR *vs* RC and O_2P-HR *vs* CS), the regression analysis indicated that the slopes were not different from unity and the intercepts were not different from zero (P>0.050), which is an indicative of high accuracy of the O_2P-HR method. On the other hand, the regression estimates of r^2 were 0.52 for comparison with RC and 0.53 for

comparison with CS, indicating a moderate precision of the O_2P-HR method. The between-animal CV was higher for the O_2P-HR method (16.6%) when compared to RC (7.7%) or CS (6.3%). The CCC were moderate, 0.70 for O_2P-HR *vs* RC and 0.73 for O_2P-HR *vs* CS. The O_2P-HR method generated EE measurements that were comparable with high accuracy and moderate precision to those estimated using RC and CS. The O_2P-HR method may presented a negative impact on power of an experiment due to its high between-animal coefficient of variation. In the same way, the FM technique for measuring methane emissions and the O_2P-HR for measuring energy expenditure of cattle are techniques that offer their advantages as compared to the most traditional techniques used. Therefore both evaluated methods should be more investigated to determine how best to deploy the systems to meet specific objectives, also to investigate ways to minimize associated errors.

RESUMO

OSS, Daniela Batista, D.Sc. Universidade Federal de Viçosa, Fevereiro de 2016. **Exigências nutricionais de energia e proteína de tourinhos mestiços (Holandês × Gir) e avaliação de técnicas para mensuração de emissão de metano e produção de calor em bovinos.** Orientador: Marcos Inácio Marcondes. Coorientador: Luiz Gustavo Ribeiro Pereira.

Os principais fatos que justificam o desenvolvimento deste trabalho são: 1 – Informações sobre exigências nutricionais de raças mestiças, largamente utilizadas no Brasil, são escassas; 2 – Uma pressão mundial para reduzir emissões de gases de efeito estufa, como metano, por ruminantes, aumentou o interesse por pesquisadores em diversas partes do mundo, em pesquisas para redução dessas emissões e consequentemente para o desenvolvimento e validação de técnicas para realizar mensurações precisas e acuradas e 3 – Considerando que o gasto energético (produção de calor) de bovinos é um dos elementos principais no orçamento da energia disponível para esses animais, a avaliação de técnicas que são aplicáveis a diferentes condições de produção são fundamentais para estudos sobre o metabolismo e partição energética de bovinos. Sendo assim, este trabalho foi desenvolvido a partir de três estudos. O primeiro foi estimar as exigências nutricionais de energia e proteína de tourinhos mestiços (Holandês × Gir). Vinte e quatro tourinhos de 10 meses de idade com peso inicial de 184.1 ± 23.36 kg (média ± desvio padrão) foram utilizados em um experimento de abate comparativo. Seis animais foram abatidos no início do experimento para determinação do peso de corpo vazio (PCVZ) dos animais e também o conteúdo inicial de proteína e energia no PCVZ dos mesmos. Os outros 18 animais foram distribuídos em um delineamento inteiramente casualizado. Os tratamentos foram 3 níveis de consumo de matéria seca (MS): 1,2% do PV, 1,8% do PV e *ad libitum*. Os tourinhos foram alimentados com uma dieta constituída por 59,6% de silagem de milho e 40,4% de concentrado, com base na MS. A relação encontrada entre PCVZ e PV demonstrou que o PCVZ corresponde a $86{,}1_{\pm 0{,}31}$% do PV dos animais, enquanto que a relação encontrada entre ganho de corpo vazio (GPCVZ) e ganho médio diário (GMD) demonstrou que o GPCVZ corresponde a $93{,}4_{\pm 1{,}11}$% do GMD dos animais. As exigências de energia líquida e metabolizável para mantença (EL_m e EM_m) observada neste estudo foi $74.8_{\pm 2.89}$ kcal/$PCVZ^{0.75}$/d e 120.8 kcal/$PCVZ^{0.75}$/d, respectivamente. A eficiência do uso da energia metabolizável para mantença ($\boldsymbol{k}_m$) observada foi de 61,9%. A equação ajustada para estimar a exigência de energia líquida para ganho (EL_g) foi:

$EL_g = 0.049_{\pm 0.0011} \times PCVZ^{0.75} \times GPCVZ^{0.729 \pm 0.0532}$. A eficiência do uso da energia metabolizável para ganho ($\boldsymbol{k}_g$) observado foi de 30,8%. A exigência de proteína metabolizável para mantença (PM_m) detectada neste estudo foi de 3.05 g/kg $PV^{0.75}$. A equação ajustada para estimar as exigências de proteína líquida para ganho (PL_g) foi: $PL_g = (87.138_{\pm 65.1378} \times GPCVZ) + (40.436_{\pm 21.3640} \times EL_g)$. A eficiência do uso da proteína metabolizável para ganho ($\boldsymbol{k}$) foi de 53,6%. Comparando as estimativas de exigências geradas neste estudo com as estimativas segundo dois diferentes sistemas de exigências nutricionais de bovinos, um que utilizou animais de raças europeias de corte como banco e outro que utilizou raças mestiças de corte (Zebu × Bos taurus) como banco de dados para gerar seus modelos, verificamos uma pequena variação nas estimativas e recomendamos o uso das exigências apresentadas neste trabalho para balancear dietas de tourinhos mestiços (Holandês × Gir). O segundo estudo teve como objetivo comparar a técnica da máscara facial (MF) para mensuração da emissão de metano por bovinos com as técnicas mais largamente e tradicionalmente utilizadas para mensuração de metano entérico: a técnica do gás traçador SF_6 e câmaras respirométricas (CR). Os mesmos animais, tratamentos e dietas que foram utilizados no estudo de exigências foram utilizados neste estudo e no estudo que será descrito posteriormente a esse. As emissões de metano foram mensuradas primeiramente utilizando a técnica do SF_6, posteriormente a técnica da máscara facial e finalmente as câmaras respirométricas, de forma não simultânea e sim consecutiva. A técnica do SF_6 foi realizada por 5 dias consecutivos, 2 semanas depois a técnica da máscara fácil foi conduzida realizando uma única medição de 30 minutos por dia, por 3 dias, para cada animal. As mensurações foram realizadas após 6 horas à alimentação dos tourinhos. Após 4 semanas, os animais foram avaliados nas câmaras respirométricas por 2 dias consecutivos. As emissões de metano (g/d) foram altamente concordantes entre as técnicas avaliadas, com coeficientes de correlação e concordância de ordem de 0,82, 0,82 e 0,74 para as comparações entre SF_6 *vs* CR, MF *vs* CR e MF *vs* SF_6, respectivamente. Quando as emissões de metano foram ajustadas para o consumo de matéria seca (CMS; g/kg CMS), as técnicas não diferiram entre si (P=0,238). No entanto, a técnica da máscara facial apresentou maiores coeficientes de variação entre dias (21,3%) e entre animais (13,4%) em rendimento de metano (g/kg CMS) comparada a técnica do SF6 (18,8% e 6,8%) e CR (12,9% e 7,5%). A técnica da máscara facial gerou resultados de produção de metano que foram comparáveis com os resultados gerados pela técnica do SF_6 e CR, no entanto devido à sua alta variação, pode apresentar limitações quando o número de replicatas

(animais) por tratamentos é baixo levando a uma diminuição do poder do experimento em detectar diferenças entre tratamentos. O terceiro estudo objetivou comparar a técnica baseada no pulso de oxigênio (O_2) e batimentos cardíacos (O_2P-BC) para estimar o gasto energético dos animais com as técnicas do abate comparativo (AC) e câmara respirométrias (CR). A técnica O_2P-BC consiste em mensurar o consumo de O_2 por períodos curtos de tempo (30 min/d) e mensurar os batimentos cardíacos por períodos de 24 horas. Para as comparações entre O_2P-BC *vs* AC e O_2P-BC *vs* CR as análises de regressão linear indicaram que os coeficientes angulares não foram diferente de 1 e que os interceptos não foram diferentes de 0 (P>0,050), o que são indicativos de uma alta acurácia do método O_2P-BC. Por outro lado, os coeficientes de determinação para as duas comparações, r^2, foi 0,52 e 0,53 entre O_2P-BC *vs* AC e O_2P-BC *vs* CR, respectivamente, indicando uma moderada precisão do método O_2P-BC. O método O_2P-BC apresentou maior coeficiente de variação (CV) entre animais (16,6%) quando comparado com CR (7,7%) e AB (6,3%). Os coeficientes de correlação e concordância foram moderados para ambas comparações: 0,70 para O_2P-BC *vs* CR e 0,73 para O_2P-BC *vs* AC. O método O_2P-BC gerou mensurações de gasto energético que foram comparáveis àqueles mensurados utilizando CR e AC, com alta acurácia e moderada precisão. O método O_2P-BC pode apresentar um impacto negativo no poder de um experimento para detectar diferenças de tratamentos devido ao seu maior CV. Igualmente, a técnica da máscara facial para mensurar metano entérico e o método O_2P-BC para estimar gasto energético de bovinos, são técnicas alternativas que oferecem suas vantagens em relação às mais tradicionais utilizadas para os objetivos citados. Devido a isso, ambas devem sofrer avaliações mais aprofundadas para determinar a melhor maneira para atender objetivos específicos e minimizar erros relacionados às técnicas em questão.

GENERAL INTRODUCTION

The animal scientific community is always investigating ways to make animal production systems more cost-effective, which is, greater performance associated with low cost production. Considering that nutrition is a key factor in performance, health and welfare of cattle, since 1959 there has been coordinated action by public organizations to review the literature on the nutrient requirements of farm livestock and update advice on feeding standards (AFRC, 1993).

Knowledge about nutrient requirements of cattle is essential to avoid excessive or insufficient nutrient supply for these animals. Energy supply is the factor that first determine animal performance. With regard to protein, the adequate supply of this nutrient is important because: it is the most expensive nutrient in animal diets and consequently, its oversupply can decrease production efficiency, and animals excrete N via feces and urine causing air and water pollution.

Nutrient requirements may vary according species, breeds, sex, age, season, temperature, physiological stage and other factors (NRC, 1996). Many nutrient requirements systems of farm animals are available, including: NRC (1996, 2001; USA) AFRC (1993; United Kingdom), INRA (1988; France), CSIRO (2007; Australia) and BR-CORTE (Valadares Filho et al., 2010; Brazil). Undoubtedly, all of them have the responsibility to move the science forward by including models in their systems that was constructed on a substantial amount of data, taking into account the factors that may influence the nutrient requirements of cattle, as mentioned above.

The BR-CORTE (Valadares Filho et al., 2010) has became fundamental for optimizing cattle performance and for lowering costs in diets formulated for Brazilian beef cattle. However, few crossbred (Holstein × Gyr) animal are included in BR-CORTE database. In several regions

of Brazil, the utilization of systems in which Holstein × Zebu animals are utilized for meat or milk production is significant, thus the nutrient requirement of these crossbred cattle become necessary to optimize the production.

Thus, studies about nutrient requirements of cattle are important make cattle raising systems more cost-effective. In a continuous attempt to achieve a greater efficiency, understanding the energy metabolism such as energy partition of cattle is also essential. According to Ferrel and Oltjen (2008), at energy partition, one of the largest energy losses of feed energy are as fecal energy and heat production (energy expenditure). Energy losses as energy expenditure may represent more than 40% of the gross energy intake (Kurihara et al., 1999), and energy losses in form of enteric methane may represent a range of 2-14% of feed energy (McAllister et al., 1996). However, the worry around methane emission from ruminants is mainly because it is a greenhouse gas, and due to that, a pressure to reduce livestock greenhouse gases has prompted greater interest in research to reduce enteric methane emissions from cattle (Martin et al., 2010; Patra, 2012).

To develop strategies to understand and to minimize energy losses as enteric methane emissions or energy expenditure it is important to have measurements techniques that are suited to different production conditions. A number of techniques are available for measuring methane emissions and energy expenditure, all with their own specific advantages and disadvantages, which will be addressed ahead.

Techniques for measuring energy expenditure of cattle

Animals expend energy, or in other words produce heat, because of metabolic reactions associated with maintenance and production metabolism and other "non-productive" functions as physical activity, thermoregulation or immune response (NRC, 1996). Measurement of the

energy expenditure may enable determination of individual efficiency; the lower the energy expenditure in relation to production of an animal the higher the efficiency of an animal, suggesting that selection of domestic ruminants for directly increased efficiency is a promising means of improving livestock efficiency (Brosh and Aharoni, 2005; Aharoni et al., 2006).

The heat production is usually measured directly by using respiration chambers. The approach is based on measuring gas exchange and changes in inhaled O_2 and exhaled CO_2 and CH_4. In this case, the Brouwer's formula (Brouwer, 1965) is often used to calculate heat production from the consumption (O_2) and the production (CO_2 and CH_4) of these gases. However, the use of indirect calorimetry allows the evaluation of animals only under controlled and closed conditions.

Another way to reach the energy expenditure of cattle is using the comparative slaughter method by which energy expenditure is estimated (measured indirectly) by the difference between the metabolizable energy intake and retained energy in the empty body weight (EBW) of the animals. The comparative slaughter allows the determination of the heat production under production conditions, however requires sacrificing animals.

Even so, significant effort has been invested to develop methods for measuring the energy expenditure of animals in their natural environmental. The O_2P-HR method is an alternative technique that allows to measure energy expenditure of cattle in the environmental where they are being raised. The O_2P-HR (oxygen pulse and heart rate) method is based on long-term measurement of heart rate and on short-term measurement of O_2P. The accuracy and precision of these two measurements are essential to the accuracy and precision of the O_2P-HR (Brosh, 2007).

The theory behind the O_2P-HR method is that the O_2 used by mammals is transported to the tissues by the heart; consequently, calibration of heart rate recording to energy expenditure may be a great candidate for use of heat production measurement of free-ranging cattle (Brosh, 2007).

The O_2P-HR method was already previously used (Arieli et al., 2002; Barkai et al., 2002) and it seems to have a great potential for agricultural application, however, further studies are needed to evaluate the method in different experimental conditions, as well as to validate its approach with the most used methods for measuring energy expenditure in cattle.

Techniques for measuring methane emissions from cattle

There are two techniques able to measure methane over the duration of an entire day: respiration chamber and the sulphur hexafluoride tracer technique (SF_6). The SF_6 technique (later modified by Deighton et al., 2014) have been widely used in several studies because, unlike the chambers, it offers the possibility for measuring methane emissions from free-ranging animals under their own environmental (Grainger et al., 2010; Hegarty et al., 2014). However, only respiration chambers are able to measure methane emitted by flatus and able to demonstrate the daily variation pattern in emissions (Crompton et al., 2010).

These two techniques were widely compared with each other, and in general, there was agreement between them across the studies (Grainger et al., 2007; Muñoz et al., 2012; Pinares-Patiño et al., 2011). Both techniques have their own strengths and weaknesses. Some of the strengths were already mentioned above, but with regard to the weaknesses, basically, respiration chambers require high capital investment and the SF_6 technique is very labor intensive.

There is an increasing interest in short-term (spot) measurements techniques to measure enteric methane from ruminants. The simplicity for obtaining short-term (spot) measurements of

methane production has caused these methods to be developed for verifying mitigation on-farm (DoE, 2013). Several methods based on short-term measurements are available, including GreenFeed (Hammond et al., 2015), ventilated hood boxes or chambers (Troy et al., 2013), portable accumulation chambers (Goopy et al., 2015), face mask system (Kawashima, 2001) and others. Short-term measurements techniques are being used but their accuracy and precision are poorly defined yet (Cottle et al., 2015). Therefore, studies of variation and comparison with the most used techniques around the world are needed for providing a basis for future application of these methods.

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CHAPTER 1

Energy and Protein Requirements of Crossbred (Holstein × Gyr) Growing Bulls

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Abstract

The objective of this study was to estimate the energy and protein requirements of crossbred (Holstein × Gyr) growing bulls. Twenty-four 10 months old bulls (initial body weight (BW) = 184 ± 23.4 kg) were used in a comparative slaughter trial. Six bulls were slaughtered at the beginning of the experiment as the reference group, in order to estimate initial empty body weight (EBW) and energy and protein contents of the remaining animals. The remaining bulls were assigned to a completely randomized design with 3 levels of DM intake, with 6 replicates. The levels of DM intake were: 1.2% of BW, 1.8% of BW and *ad libitum* – target orts 5%. The remaining bulls were slaughtered at the end of the experiment. Bulls were fed a diet consisting of 59.6% corn silage and 40.4% concentrate on a DM basis. The equation adjusted that demonstrate the relationship between EBW and BW was $EBW = 0.861_{\pm 0.0031} \times BW$. While the relationship between empty body gain (EBG) and average daily gain (ADG) may be demonstrated using the equation: $EBG = 0.934_{\pm 0.0111} \times ADG$. The net energy requirements for maintenance (NE_m) was $74.8_{\pm 2.89}$ kcal/$EBW^{0.75}$/d, the metabolizable energy requirements for gain (ME_m) was 120.8 kcal/$EBW^{0.75}$/d. The efficiency of the use of metabolizable energy for maintenance (k_m) detected was 61.9%. The equation adjusted to estimate the net energy for gain (NE_g) was: $NE_g = 0.049_{\pm 0.0011} \times EBW^{0.75} \times EBG^{0.729 \pm 0.0532}$. The efficiency of the use of metabolizable energy for gain (k_g) observed was 30.8%. The metabolizable protein requirements for maintenance (MP_m) was 3.05 g/kg $BW^{0.75}$. The equation adjusted to estimate the net protein requirements for gain was: $NP_g = (87.138_{\pm 65.1378} \times EBG) + (40.436_{\pm 21.3640} \times NE_g)$. The efficiency of the use of metabolizable protein for gain (k) observed was 53.6%. We conclude that the estimates of energy and protein requirements herein presented is more appropriate when compared to other systems to balance diets of crossbred (Holstein × Gyr) growing bulls.

Introduction

Experiments that seek estimate nutrient requirements of specific breeds may be justified due to the need of increasing the database to enable new meta-analysis to allow updates and validations of more consistent models. Undoubtedly, all nutrient requirements systems around the world have the responsibility to move the science forward by including models in their systems that was constructed on a substantial amount of data.

The *Bos taurus indicus* cattle and their crossbreds are commonly used in dairy and beef production in tropical regions. The objective of using *Bos taurus indicus* for crossbreeding (*Bos taurus indicus* × *Bos taurus taurus*) is to overcome limitations regarding hot and humid weather, intense sunshine, parasites and utilization of poor quality forages [1].

The dairy Gyr (*Bos taurus indicus*) breed has mainly been selected for milk yield and it is found throughout in countries such as Brazil. The desirable characteristics in dairy Gyr have raised the interest of other tropical countries, resulting in the exportation of semen and animals to Africa and Central and South America [2]. In Brazil for example, about 70% of milk production comes from crossbred (Holstein × Zebu) cows, being the most common crossbreeding Holstein × Gyr [3].

Surely, the Nutrient Requirements of Dairy Cattle [4] is the most widely system used to balance diets for dairy cattle. However, the equations to estimate energy and protein requirements presented by this system for growing animals are from a study involving mostly beef British breeds [5] which was the same adopted by the Nutrient Requirements of Beef Cattle [6]. The Nutrient Requirements of Dairy Cattle [4] adopted the same equations because they were validated using data from experiments with Holstein in growth stage, also because these equations accurately described the net and protein content of Holstein growing animals [7]. Since

then, it seems that experiments seeking estimating nutrient requirements of dairy growing cattle have faded.

In an attempt to provide information regarding nutrient requirements of cattle used in tropical regions (mainly Nellore and Nellore × *Bos taurus taurus*), Brazilian researchers published a nutrient requirements system for beef cattle, the BR-CORTE [8]. Although this was a great step taken by Brazilian researchers, and considering that crossbred Holstein × Gyr cattle is very representative in countries including Brazil, and others from Africa and Central and South America, it is undeniable that estimates of nutrient requirements of this crossbreeding is still a gap.

Therefore, we believe that determining the nutrient requirements of crossbred cattle (Holstein × Gyr) and providing appropriate knowledge to balance diets is absolutely necessary to avoid excessive or insufficient nutrient supply for these animals [9]. In addition, information about requirements of Holstein × Gyr is scarce in the literature [10, 11]. Taking into account that the greater operational cost of production in meat or dairy production is due to nutrition, it can generate a big economic impact in cattle production systems in tropical conditions once the Holstein × Gyr cattle are very representative in the meat and dairy market.

Given these reasons, the objective of this study was to estimate energy and protein requirements for maintenance and gain of crossbred (Holstein × Gyr) growing bulls.

Materials and Methods

Animal care procedures throughout the study followed protocols approved by the Conselho de ética no uso de animais de produção (CEUAP/UFV; process number 044/2012) of the Universidade Federal de Viçosa. The experiment was conducted at the Multi-use Complex on

Livestock Bioefficiency and Sustainability at the Embrapa Gado de Leite (Embrapa Dairy Cattle), in Coronel Pacheco, MG, Brazil, from August 2013 to February 2014.

Animals, diet and experimental design

Twenty-four Holstein × Gyr crossbred 10 months old bulls (initial body weight = 184 ± 23.4 kg) were used. Initially, all bulls were treated for ectoparasites and endoparasites (Ivomec, Paulina, São Paulo, Brazil). The bulls were randomly subdivided into 4 groups of 6 animals at the beginning of the experiment. One of those groups was designated as baseline reference group, and it was slaughtered at the beginning of the experiment to estimate initial body weight (EBW) and initial body energy and protein content of the other animals. The 3 remaining groups were fed at 3 levels of DM intake: (1) 1.2% of BW (close to maintenance level); (2) 1.8% of BW or (3) *ad libitum* – target 5% orts. These treatments were chosen intending to achieve a variability in the metabolizable energy and protein intake (MEI and MPI) to obtain a better model's adjustment. One animal from the *ad libitum* group had to be removed from the experiment due to health issues. Bulls were housed in a tie stall system with free access to water.

The diet was formulated according to BR-CORTE [8] for an average daily gain (ADG) of 1.2 kg/d. Throughout the study, bulls were fed a diet consisting of corn silage and concentrate (59.6: 40.4 DM basis) once daily. The concentrate was composed of: soybean meal, ground corn, urea, mineral mix and limestone (Table 1). The feed ingredients of the concentrate were collected for analysis at the time concentrate was manufactured. Representative samples of silage, concentrate and orts were collected daily, and pooled monthly for chemical analysis (Table 2). The composite sample of orts was made for each month proportionally to weight (DM

basis) from each day. Feed DM offered and refused were weighed to determine total daily dry matter intake (DMI).

Table 1. Composition of the concentrate and experimental diet (g/kg; DM basis)

Ingredient	Concentrate	Diet
Corn silage		596
Ground corn	679	274.3
Soybean meal	248	100.2
Mineral mix[a]	34.9	14.1
Urea	24	9.7
Limestone	14.1	5.7
Total	1000	1000

[a]Guarantee levels: Ca: 200 g/kg min; P: 60 g/kg; Mg: 20 g/kg; K: 35 g/kg; S: 20 g/kg; Na: 70g/kg; Co: 15 mg/kg; Cu: 700 mg/kg; Mn: 1600 mg/kg; Zn: 2500 mg/kg; Se: 19 mg/kg; I: 40 mg/kg

Table 2. Chemical composition of the ingredients used in experimental diets (g/kg; DM basis)

Item	Corn silage	Concentrate	Diet
Dry matter	307.2	891.8	543.4
Organic matter	947.7	924.8	938.4
Crude protein	77.3	252.5	148.1
Ether extract	24.4	35.1	28.8
NDF[a]	464.6	145.8	335.8
NFC[b]	381.4	491.3	425.8
GE[c] (Mcal/kg)	4.3	4.3	4.3

[a]Neutral detergent fiber; [b]Non-fibrous carbohydrates; [c]Gross energy

The samples were analyzed for contents of dry matter (DM; [12] method 930.15), ash ([12] method 924.05), crude protein (CP; [12] method 984.13), ether extract (EE; [12] 1990; method 920.39), neutral detergent fiber (NDF; [13]) with heat stable amylase and expressed exclusive of residual ash, and non-fibrous carbohydrates (NFC; [14]). Gross energy was determined using an adiabatic calorimeter (model C-5000, Labcontrol IKA, São Paulo, SP). The bulls were weighed at 15 d intervals at 0730 AM before feeding (0830 AM) to calculate ADG. The animals from the 3 remaining groups were slaughtered at the end of the experiment to determine their final EBW and final body energy and protein content. The experiment lasted 173 d, 171 d and 168 d for the groups 1.2% of BW, 1.8% of BW and *ad libitum* respectively, when the slaughter was performed.

Digestibility trial

Two digestibility trial were conducted at 2 points throughout the experiment: 2 months after the reference slaughter and 2 months before the final slaughter. The total feces and urine were collected for three consecutive days from all animals from the remaining groups [15]. 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 analysis that were performed for experimental diet were performed for feces to calculate the DM, nutrients and energy digestibility coefficients. Metabolizable energy content was determined by multiplying digestible energy by 0.82 [6].

The urine collection was performed by using collecting rubber funnels attached to the bulls. Each funnel had a hose to carry the urine to an individual polyethylene container containing 20% H_2SO_4 intending to reduce N and purine derivative losses. Also, each container was kept immersed in ice. After each 24 h collection day, the total urine excreted was weighed and measured. The contents of each container were homogenized and a 50 mL sample was taken and stored at -20^{o}C for further laboratory analysis.

A nitrogen balance was performed at each digestibility trial to estimate digested nitrogen (DN) and retained nitrogen (RN). The DN was calculated as the difference between N intake and fecal N, while the RN was calculated as the difference between the DN and the urinary nitrogen.

The metabolizable protein intake (MPI) was calculated as the sum of the true microbial protein digestible and the digestible ruminally undegraded protein (DRUP) intakes. The microbial protein production was calculated from the purine derivative excretion that is the sum of total allantoin and uric acid excretions, which were obtained by multiplying their

concentrations by the daily urinary volume [16]. The true fraction and digestibility adopted for microbial protein was 80% and 80%, respectively [4]. The ruminally undegraded protein (RUP) intake was calculated as the difference between the crude protein intake and the rumen-degradable protein (RDP) intake. The digestibility adopted for RUP was 80%. The RDP intake was calculated taking into account an efficiency of N utilization in the rumen of 90% [4].

Slaughter and sampling

All bulls were fasted for 16 h to obtain the shrunk body weight (SBW) before slaughter. Bulls from the same group were slaughtered on the same day. Bulls were slaughtered by using captive bolt stunning followed by bleeding. The blood was sampled at the moment of bleeding to avoid sampling after coagulation. After bleeding, the gastrointestinal tract was removed and it was washed out to winkle out all digesta. The heart, lungs, liver, spleen, kidneys, the fat around the kidney, pelvis and heart (KPH fat), diaphragm, mesentery, tails, trimmings, and cleaned gastrointestinal tracts were weighed. The carcasses, head, leather, limbs and blood were also weighed to determine the EBW.

The rumen, reticulum, omasum, abomasum, small and large intestines, KPH fat, mesentery, liver, heart, kidneys, lung, tongue, spleen, diaphragm, esophagus, trachea, tails and reproductive tract of each bull were homogenized in an industrial cutter for 20 min. A sample then was taken to compose a “sample of organs and viscera”.

After removing the leather, the head and limbs were ground in a bone crusher for 20 min. A sample then was taken to compose a “sample of head and limbs”. The leather of each animal was sampled in two parts to represent the shoulder, three parts to represent the dorsal line, two parts to represent the ventral line, two parts to represent the rear, one part to represent each foot,

and one part to represent the head, which altogether represented the entire leather [17]. All parts of the leather were minced in about 2 cm^2 small pieces. A sample then was taken to compose a "sample of leather".

After slaughter, the carcasses of each animal were split into two half-carcasses which were chilled at 4°C for 18 h. After the 18 h-period, the left half-carcass was ground in a bone crusher for 20 min and it was transferred to an industrial cutter to be homogenized for 20 min. After homogenization, a sample was taken to compose a "sample of carcass".

All samples from each animal (blood, organs and viscera, head and limbs, leather and carcass) were previously lyophilized before to be ground in a knife mill (2 mm) and after in an industrial blender by adding liquid nitrogen. A composite sample of the whole animal was made by using relative individual proportion (DM basis) in the EBW. It was homogenized by using an industrial blender by adding liquid nitrogen for further laboratory analysis.

The DM, CP, EE, ash, and GE contents were determined on the composite sample of the whole animal, following the methods described above for experimental diet ingredients and feces.

Procedures used to verify relationship among live, shrunk and empty body weight and between average daily gain and empty body gain

To verify the relationship among live, shrunk and empty body weight a linear regression was performed as follows:

[Equation 1]

$$\text{EBW or SBW} = \beta_0 + (\beta_1 \times SBW \text{ or } BW)$$

Where EBW is empty body weight (kg), SBW is shrunk body weight (kg) and BW is live body weight (kg) and β_0 and β_1 are regression parameters.

To verify the relationship between average daily gain and empty body gain a linear regression was performed as follows:

[Equation 2]

$$\text{EBG} = \beta_0 + (\beta_1 \times ADG)$$

Where EBG is empty body gain (kg/d), ADG is average daily gain (kg/d) and β_0 and β_1 are regression parameters.

Procedures used to estimate energy requirements

To estimate the net energy requirements for maintenance (NE_m) it was used a non-linear exponential model to describe the relationship between heat production (HP) and metabolizable energy intake (MEI) according to the model [18]:

[Equation 3]

$$\text{HP} = \beta_0 \times e^{\beta_1 \times \text{MEI}}$$

Where HP is the heat production (Mcal/$EBW^{0.75}$), MEI is the metabolizable energy intake (Mcal/$EBW^{0.75}$), and β_0 and β_1 are regression parameters. Under this model, β_0 represents the NE_m.

By the iterative method, the point where MEI equals to HP can be determined, and this point is considered the metabolizable energy requirement for maintenance (ME_m).

Heat production (Mcal/$EBW^{0.75}$) was calculated as the difference between MEI (Mcal/$EBW^{0.75}$) and retained energy (RE; Mcal/$EBW^{0.75}$). The MEI was determined as described

above in Digestibility trial section and the RE was determined as the difference between the final energy content and initial energy content in the EBW.

The efficiency of use of metabolizable energy for maintenance (k_m) was obtained from the relation between the NE_m and the ME_m [6], by the following:

[Equation 4]

$$k_m = \frac{\mathrm{NE_m}}{\mathrm{ME_m}}$$

To predict the net energy requirements for gain (NE_g) the following model was used [19]:

[Equation 5]

$$\mathrm{RE} = \beta_0 \times EBW^{0.75} \times EBG^{\beta_1}$$

Where RE is the retained energy (Mcal/d), $EBW^{0.75}$ is the metabolic empty body weight, EBG is the empty body weight gain (kg/d) and β_0 and β_1 are regression parameters. Under this model, RE represents the NE_g.

To estimate the metabolizable energy requirements for gain (ME_g), it was needed to estimate the efficiency of the use of metabolizable energy for gain (k_g). The k_g was estimated as the slope of the regression of RE on the MEI for gain as the following model [20]:

[Equation 6]

$$\mathrm{RE} = \beta_0 + (\beta_1 \times MEI_g)$$

Where RE is retained energy (Mcal/$EBW^{0.75}$/d), MEI_g is metabolizable energy intake for gain (Mcal/$EBW^{0.75}$/d) which was estimated as the difference between total MEI (Mcal/$EBW^{0.75}$/d) and MEI_m (Mcal/$EBW^{0.75}$/d) estimated as described above, and β_0 and β_1 are regression parameters. Under this model, β_1 represents the k_g.

The ME_g was then obtained from the relation between the NE_g and the ME_g, by the following:

[Equation 7]

$$\mathrm{ME}_g = \frac{\mathrm{NE_g}}{\boldsymbol{k}_\mathrm{g}}$$

Procedures used to estimate protein requirements

The metabolizable protein for maintenance (MP_m) was estimated by the relationship between the intercept (β_0) from the linear regression between the metabolizable protein intake (MPI) and the empty body gain (EBG) as following [21]:

[Equation 8]

$$\mathrm{MPI} = \beta_0 + (\beta_1 \times EBG)$$

Where MPI is metabolizable protein intake (g/d), EBG is empty body gain (kg/d), and β_0 and β_1 are regression parameters. The MP_m was then estimated by the relation between the β_0 and the average metabolic body weight ($BW^{0.75}$) as following [6]:

[Equation 9]

$$\mathrm{MP}_m = \frac{\beta_0}{BW^{0.75}}$$

Where MP_m is the metabolizable protein requirements for maintenance (g/kg $BW^{0.75}$), β_0 is the parameter determined from the Equation 8 and $BW^{0.75}$ is the average metabolic body weight.

The net protein requirements for gain (NP_g) was estimated by using a model involving EBG and RE, as following [6, 8]:

[Equation 10]

$$\mathrm{RP} = (\beta_0 \times EBG) + (\beta_1 \times RE)$$

Where RP is retained protein (g/d); EBG is empty body gain (kg/d), RE is retained energy (Mcal/d) and and β_0 and β_1 are regression parameters.

The efficiency of the use of metabolizable protein for gain (k) was estimated by the linear relationship between retained nitrogen (RN) and digested nitrogen (DN) according to the following model:

[Equation 11]

$$\text{RN} = \beta_0 + (\beta_1 \times DN)$$

Where RN is retained nitrogen (g/d), DN is digested nitrogen (g/d) and β_0 and β_1 are regression parameters. The RN and DN were determined as described above in Digestibility trial section. The RN and DN from both digestibility trial were pooled together to analyze the linear relationship between them once there was no statistical difference between the RN and DN from both digestibility trials. Under the Equation 11, the β_1 is the efficiency of the use of metabolizable protein for gain (k).

The relation between the NP_g and the k was used to estimate the metabolizable protein requirements for gain (MP_g):

[Equation 12]

$$MP_g = \frac{\text{NP}_\text{g}}{\boldsymbol{k}}$$

Where MP_g is the metabolizable protein requirements for gain (g/kg $EBW^{0.75}$/d), NP_g is the net protein requirements for gain (g/kg $EBW^{0.75}$/d) and $\boldsymbol{k}$ is the efficiency of the use of metabolizable protein for gain.

Statistical procedures

The DM, nutrients and energy intake and digestibility coefficients were analyzed using a mixed model (PROC MIXED of SAS Inst. Inc., Cary, NC) where digestibility trail was considered random effect in the model and treatments as fixed effects. The level of 0.05 was used as critical level of probability and the Tukey-Kramer test was used to detect difference between treatments.

The models described above were fit as linear and non-linear models built by the feature PROC MIXED and PROC NLIN of SAS respectively. The data analyzed as non-linear models were adjusted by the Gauss-Newton method. For all models, outliers were removed when the studentized residuals were greater than |2|, and 0.05 was used as critical level of probability to verify the significance of parameters of the models.

Results

The DM, nutrients and energy intake and digestibility coefficients

The DM, nutrients and energy intake were different among treatments (Table 3). The treatment 1.2% of BW presented greater DM, nutrients and energy digestibility coefficients when compared to treatments 1.8% of BW and *ad libitum.* The treatment 1.8% of BW presented greater DM, OM, NFC and GE digestibility coefficients compared to *ad libitum* treatment (Table 4).

Table 3. Least square means, standard error and significance of effects for nutrient (kg) and energy (Mcal) intake according to levels of DM supply

Item	DM intake level			SE	*P*-value
	1.2 % of BW (*n*=6)	1.8% of BW (*n*=6)	*Ad Libitum* (*n*=5)		
DMI[a]	2.20[c]	4.24[b]	7.66[a]	0.584	0.001
OMI[b]	2.06[c]	3.97[b]	7.18[a]	0.361	0.001
CPI[c]	0.33[c]	0.64[b]	1.19[a]	0.056	0.001
EEI[d]	0.06[c]	0.12[b]	0.23[a]	0.011	0.001
NDFI[e]	0.72[c]	1.38[b]	2.47[a]	0.133	0.001
NFCI[f]	0.94[c]	1.83[b]	3.30[a]	0.161	0.001
TDNI[g]	1.64[c]	2.95[b]	5.07[a]	0.270	0.001
GEI[h]	9.38[c]	18.08[b]	32.67[a]	1.645	0.001
DEI[i]	6.91[c]	12.38[b]	21.19[a]	1.552	0.001
MEI_j	5.67[c]	10.15[b]	17.37[a]	1.273	0.001

[a]dry matter intake; [b]organic matter intake; [c]crude protein intake; [d]ether extract intake; [e]neutral detergent fiber intake; [f]non-fiber carbohydrates intake; [g]total digestible nutrients intake; [h]gross energy intake; [i]digestible energy intake; [j]metabolizable energy intake

a-c Means within intake level in a row followed by different letters differ (P≤0.05)

Table 4. Least square means, standard error and significance of effects for nutrient (g/kg) and energy (kcal/Mcal) digestibility coefficients according to levels of DM supply

Item[a]	DM intake level			SE	*P*-value
	1.2 % of BW (*n*=6)	1.8% of BW (*n*=6)	*Ad Libitum* (*n*=5)		
DM	744[a]	698[b]	659[c]	9.6	0.001
OM	760[a]	712[b]	674[c]	9.3	0.001
CP	727[a]	691[b]	679[b]	11.6	0.001
EE	886[a]	829[b]	808[b]	21.3	0.001
NDF	632[a]	540[b]	514[b]	16.6	0.001
NFC	860[a]	841[a]	781[b]	10.3	0.001
GE	737[a]	685[b]	648[c]	10.7	0.001

[a]Digestibility coefficients of: DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; NFC = non-fiber carbohydrates and GE = gross energy

a-c Means within intake level in a row followed by different letters differ (P≤0.05)

Energy and protein requirements

The descriptive database of the bulls that were used in this study is shown in Table 5. The summary of all equations fit for estimating the total requirements of energy and protein of dairy crossbred bulls in this study is presented in Table 6.

Table 5. Descriptive of the database (mean ± SD) used to obtain the energy and protein requirements of crossbred (Holstein × Gyr) growing bulls

Item	DM intake level		
	1.2 % of BW (n = 6)	1.8% of BW (n = 6)	*Ad Libitum* (n = 5)
$BW_{initial}$[a] (kg)	181.5 ± 22.42	187.8 ± 29.32	182.6 ± 21.13
BW_{final}[b] (kg)	190.5 ± 24.71	281.3 ± 50.62	388.2 ± 48.77
$EBW_{initial}$[c] (kg)	151.6 ± 18.73	156.9 ± 24.49	152.5 ± 17.64
EBW_{final}[d] (kg)	166.3 ± 18.62	244.6 ± 44.43	343.8 ± 46.00
DMI[e] (%BW)	1.25 ± 0.027	1.90 ± 0.034	2.76 ± 0.188
ADG[f] (kg/d)	0.05 ± 0.061	0.55 ± 0.130	1.22 ± 0.232
EBG[g] (kg/d)	0.09 ± 0.039	0.51 ± 0.128	1.14 ± 0.229
RE[h] (Kcal/$EBW^{0.75}$)	10.0 ± 3.54	28.8 ± 5.16	54.8 ± 8.45
MEI[i] (Kcal/$EBW^{0.75}$)	133.3 ± 5.17	199.8 ± 9.35	285.0 ± 23.14
HP[j] (Kcal/$EBW^{0.75}$)	123.3 ± 4.59	171.0 ± 9.44	230.1 ± 25.12
RP[k] (g/$EBW^{0.75}$)	37.1 ± 9.38	120.1 ± 28.25	232.2 ± 44.29
PMI[l] (g/$EBW^{0.75}$)	5.1 ± 0.18	8.1 ± 0.48	12.8 ± 1.42
RE_f[m] (%)	52.7±11.93	58.1±7.51	64.2±2.75
RE_p[n] (%)	47.3±11.93	41.9±7.51	36.1±2.75
EBG_p[o] (g/kg EBW)	605±27.7	561±28.5	519±30.7
EBG_f[p] (g/kg EBW)	236±29.0	304±44.3	395±25.1

[a]$BW_{initial}$ = initial body weight; [b]BW_{final} = final body weight; [c]$EBW_{initial}$ = initial empty body weight; [d]EBW_{final} = final empty body weight; [e]DMI (%BW) = dry matter intake in percentage of BW; [f]ADG = average daily gain; [g]EBG = empty body weight gain; [h]RE = retained energy; [i]MEI = metabolizable energy intake; [j]HP = heat production; [k]RP =

retained protein; [l]PMI = protein metabolizable intake; [m]RE_f = percentage of RE deposited as fat; [n]RE_p = percentage of RE deposited as protein; [o]EBG_f = proportion of fat in EBG; [p]EBG_p = proportion of protein in EBG.

Table 6. Abstract of estimative models of energy and protein requirements of crossbred (Holstein × Gyr) growing bulls

Item	Equation	Unit
SBW	0.976 × BW	kg
EBW	0.882 × SBW	kg
EBW	0.861 × BW	kg
EBG	0.934 × ADG	kg/d
	Energy requirements	
NE_m	$0.075 \times EBW^{0.75}$	Mcal/d
k_m	61.9	%
ME_m	$0.121 \times EBW^{0.75}$	Mcal/d
NE_g	$0.049 \times EBW^{0.75} \times EBG^{0.729}$	Mcal/d
k_g	30.8	%
ME_g	NE_g / K_g	Mcal/d
ME_t	$ME_m + ME_g$	Mcal/d
TDN	$ME_t/0.82/4.409$	kg/d
	Protein requirements	
MP_m	$3.05 \times BW^{0.75}$	g/d
NP_g	$(87.138 \times EBG) + (40.436 \times NE_g)$	g/d
k	53.6	%

MP_g	NP_g/k	g/d
MP_t	$MP_m + MP_g$	g/d
MicP	110 × TDN	g/d
RDP	1.11 × MicP	g/d
RUP	[(MPt – (MicP × 0.64)]/0.80	g/d
CP	RDP + RUP	g/d

Relationship among live, shrunk and empty body weight and between average daily gain and empty body gain

The equation adjusted from the relationship between SBW and BW was (MSE=19.009; r^2=0.997):

[Equation 13]

$$\text{SBW} = 0.976_{\pm 0.0061} \times \text{BW}$$

The equation adjusted from the relationship between EBW and SBW was (MSE=51.179; r^2=0.942):

[Equation 14]

$$\text{EBW} = 0.882_{\pm 0.0061} \times \text{SBW}$$

The equation adjusted from the relationship between BW and EBW was (MSE=8.720; r^2=0.972):

[Equation 15]

$$\text{EBW} = 0.861_{\pm 0.0031} \times \text{BW}$$

Similarly, the intercept of the linear regression between ADG and EBW was not different of zero (P=0.0981) and the equation adjusted from the relationship between ADG and EBG was (MSE=0.001; r^2=0.973):

[Equation 16]

$$\mathrm{EBG} = 0.934_{\pm 0.0111} \times \mathrm{ADG}$$

Energy requirements

The NE_m observed in our study was 74.8 kcal/$EBW^{0.75}$/d according to the following equation (MSE=0.00005; r^2=0.969):

[Equation 17]

$$\mathrm{HP} = 0.075_{\pm 0.0029} \times e^{3.968_{\pm 0.1597} \times \mathrm{MEI}}$$

Through iterative method the ME_m was determined as 120.8 kcal/$EBW^{0.75}$/d, and $\boldsymbol{k}_m$ was 61.9%.

To estimate the NE_g the following equation was adjusted (MSE=0.044; r^2=0.991):

[Equation 18]

$$NE_g = 0.049_{\pm 0.0011} \times EBW^{0.75} \times EBG^{0.729_{\pm 0.0532}}$$

The $\boldsymbol{k}_g$ observed in this study was 30.8% (Fig 1).

Fig 1. The relationship between retained energy (RE) and metabolizable energy intake for gain (MEI_g) of Holstein × Gyr crossbred growing bulls. This figure shows the linear relationship between retained energy and metabolizable energy intake for gain (MEI_g) of Holstein × Gyr crossbred growing bulls, where the efficiency of use of metabolizable energy for

gain (k_g) is the slope of the regression. The equation adjusted was: RE = $0.006_{\pm 0.0026}$ + $0.308_{\pm 0.0284}$ × MEI_g (MSE=0.00004; r^2=0.894;).

Protein requirements

The parameter β_0 observed from the Equation 8 was 181.0 ± 27.8. Then, the equation adjusted to estimate the MP_m was:

[Equation 19]

$$MP_m = \frac{181.0_{\pm 27.8}}{BW^{0.75}}$$

As the average $BW^{0.75}$ was 59.3 ± 9.82 kg, thus the MP_m estimated in this study was 3.05 g/$BW^{0.75}$/d.

The equation adjusted to estimate the NP_g was (MSE=314.89; r^2=0.903):

[Equation 20]

$$NP_g = (87.138_{\pm 65.1378} \times EBG) + (40.436_{\pm 21.3640} \times NE_g)$$

The ***k*** observed in this study was 53.6% (Fig 2).

Fig 2. The relationship between retained nitrogen (RN; g/d) and digested nitrogen (DN; g/d) of Holstein × Gyr crossbred growing bulls. This figure shows the linear relationship between retained nitrogen (RN; g/d) and digested nitrogen (DN; g/d) of Holstein × Gyr crossbred growing bulls where the efficiency of use of metabolizable protein for gain (*k*) is the slope of the regression. The equation adjusted was: RN = $-16.525_{\pm 3.9685}$ + $0.5358_{\pm 0.0489}$ × DN (MSE=90.999; r^2=0.878).

Discussion

The DM, nutrients and energy intake and digestibility coefficients

Naturally, animals under restricted DM supply when compared to *ad libitum* treatment presented lower nutrients and energy intake (P=0.001; Table 3).

The DM, OM, CP, EE, NDF, CNF and energy digestibility coefficients were 12.9%, 12.8%, 7.1%, 9.7%, 22.9%, 10.1%, and on average 13.7% greater for the 1.2% of BW treatment (maintenance) when compared with the *ad libitum* treatment. The decrease in digestibility according to the increase of DM intake was previously reported [22]. This occurs due to a higher ruminal digesta passage rate [23], which results in a short time available to digestion by the microbial population in the rumen [24]. When the level of intake is decreased, as occurred for maintenance treatment (1.2% of BW), there is a greater retention time of particle in the rumen and in total gastrointestinal tract, besides a reduction in the average size of ruminal and rectal particle [25].

Relationship among live, shrunk and empty body weight and between average daily gain and empty body gain

The average ratio between SBW and EBW in this study was 0.882, value that was close to those reported by the BR-CORTE [8]: 0.895 for beef *Bos taurus indicus* cattle and their croosbreds. However, this relationship may vary between 85% and 95% [6]. The NRC dairy cattle [4] sets that EBW is 0.891 of SBW and 0.855 of BW.

The average ratio between ADG and EBG was 0.934, while the ratio reported by the BR-CORTE [8] was 0.966 for crossbred cattle (Nellore × *Bos taurus taurus*) and the ratio reported by the NRC dairy cattle [4] and CSIRO system [26] were 0.960 and 0.917 respectively.

Energy requirements

In our study the NE_m observed was 74.8 kcal/$EBW^{0.75}$. The NE_m requirements of beef cattle (British breeds) have been estimated as 77 kcal/$EBW^{0.75}$ [6, 18] while the NRC dairy cattle [4] uses a value of 80 kcal/$BW^{0.75}$. The NE_m requirements of *B. indicus* proposed by the NRC beef cattle [6] is 70 kcal//$EBW^{0.75}$. The NE_m reported by the BR-CORTE [8] for crossbred cattle was 74.2 kcal/$EBW^{0.75}$. Although the BR-CORTE's database contained only 7 animals (of 753) Nellore × Holstein, its reported NE_m is very similar to ours.

It was latter observed for finishing Holstein × Zebu bulls a NE_m equal to 78.7 kcal/$EBW^{0.75}$ [11] what is coherent once as animals grow, the energy requirements increase. The NE_m requirements of crossbred Holstein × Gyr bulls estimated in this study is intermediate to those observed for purebred cattle (*Bos taurus indicus* or *Bos taurus taurus*) and slightly lower than that indicated for growing dairy cattle by the NRC dairy cattle [4]. It is possible to observe (S1 Spreadsheet) that our result generated an intermediate value for estimates of NE_m when compared to the estimates generated from the NRC dairy and beef cattle [4, 6] and the BR-CORTE [8]. This suggests that dairy crossbreds *Bos taurus taurus* × *Bos taurus indicus* present a higher requirement than beef crossbreds *Bos taurus taurus* × *Bos taurus indicus* but a lower requirement when compared to purebreds.

The ME_m estimated in this current study was 120.8 kcal/$EBW^{0.75}$ and the k_m was 61.9%, while another study that also evaluated Holstein × Zebu cattle reported 114.2 kcal/$EBW^{0.75}$ and

68.9% [11]. When the k_m was calculated by using the equation that take into account the effects of the k_g and the EBG on the k_m [27], the k_m was very close to that estimated from the bulls of this study, 60.6%. The variability in estimates of NE_m may be related partly to differences in mathematical models and accuracy of measurements [28, 29]. Also, the NE_m is influenced by the physiological conditions of age, gender, physical activity and temperature [6]. The k_m detected in this study was very similar compared to those recommend by the NRC dairy and beef cattle [4, 6] and lower when compared to that recommended by the BR-CORTE [8] for beef crossbred *Bos taurus taurus* × *Bos taurus indicus*, what indicate that the use of the k_m of this current study or the k_m recommended by the NRC dairy cattle [4] are better recommend for dairy crossbred cattle.

With regard to the NE_g, the exponent of EBG observed in this study (0.729) is lower than that reported by BR-CORTE [8] for bulls (1.095) and that reported by NRC [4] for growing dairy cattle (1.097). However the intercept for determining NE_g in our study (0.049) is close to that reported by BR-CORTE [8] for bulls (0.053) but slightly lower than that reported by NRC dairy cattle [4] for growing animals: 0.064.

In this study, bulls were slaughtered with relatively low body weight (Table 3), besides being submitted to less energetic diets (below 50% of concentrate in the diet). Energy content and protein content of the gain at a particular EBG increases and decreases respectively, with weight in a particular body size [19]. Thus, this lower exponent of EBG may suggest that there would be a lower fat concentration in the gain of the bulls, suggesting that diet and growth stage provided a greater protein proportion in the EBG. Our results indicating a greater proportion of protein than fat in EBG (g/kg EBG; EBG_p and EBG_f) corroborate with this hypothesis (Table 3). As occurred to NE_m, the NE_g of crossbred Holstein × Gyr was intermediate (S1 Spreadsheet) when compared to the NRC dairy and beef cattle [4, 6] and BR-CORTE [8].

Naturally, the partial efficiency of energy utilization for production is lower that it is for maintenance [30]. The k_g observed in this study was 30.8% (Fig 1). This value is much lower than the k_g found in another study that evaluated Zebu × Holstein bulls fed high and low energy diets: 46.3% [11]. Also, it is lower than those reported by NRC dairy and beef cattle [4, 6] and by the BR-CORTE [8]: 35% for low energy diets and 47% for high energy diets (below or above 50% of concentrate in the diet). The k_g depends on the proportions of retained energy in the form of protein and fat thus, an estimate of k_g should take into account the gain composition. Thus, when the k_g is calculated as proposed by the BR-CORTE [8] the k_g increased from 30.8% to 34.4% indicating that there was an effect of the percentage of protein deposited as retained energy on k_g.

Protein requirements

In this study the MP_m found was 3.05 g of protein/$BW^{0.75}$. The NRC beef cattle [6] adopted a value of 3.80 g of protein/$BW^{0.75}$ for MP_m from the Wilkerson's study [21]. The MP_m adopted by the INRA system [31] and by the AFRC system [32] were 3.25 and 2.30 g of protein/$BW^{0.75}$, respectively. The MP_m found for Holstein × Gyr bulls in another study was 2.72 g of protein/$BW^{0.75}$ [11]. Although our result for MP_m shows to be close to these previous discussed, it generated a lower estimate of MP_m when compared to other systems, as commented previously in our discussion, thus we recommend that our estimates of MP_m should be used for crossbred (Holstein × Gyr) growing cattle (S1 Spreadsheet).

Regarding the NP_g, taking into account a bull weighting 250 kg and with 1 kg ADG, the NP_g estimated from the equation adjusted in this study (Eq. 20) was about 187 g/d (S1 Spreadsheet). This NP_g was higher when compared to that suggested by the BR-CORTE for

Nellore × *Bos taurus taurus* bulls [8] and the NRC dairy and beef cattle [4, 6]. This result is coherent once these both systems have used finishing cattle (>300 kg) to generate their estimates of protein requirements whereas in our study animals were in the growth stage (186 to 285 kg, on average).

The *k* observed in this study was 53.6%, which was about 14% higher than the *k* proposed by the BR-CORTE [8] which was 46.9% for animals with SBW heavier than 350 kg, but about 11% lower when animals has a SBW lighter than 350 kg. On the other hand, it was slightly similar to that *k* recommend by the NRC dairy cattle [4] for heifers or steers with equivalent SBW greater than 478 kg (S1 Spreadsheet). The *k* may be most related to the quality of metabolizable protein [33].

Conclusions

Overall, the estimates of energy requirements of crossbred Holstein × Gyr growing bulls were intermediate when compared to those estimated from a system that used beef British purebreds (also used for Holstein), and to a most recent system used for beef crossbreds (*Bos taurus taurus* × *Bos taurus indicus*), NRC and BR-CORTE, respectively. On the other hand, the estimates of protein requirements were, in general, moderately higher than those systems above mentioned. Thus, we recommend the use of the estimates of energy and protein requirements herein presented to balance diets for growing Holstein × Gyr bulls.

This current study evaluating estimates of energy and protein requirements of crossbred (Holstein × Gyr) cattle is one of few that exist in the literature. Given these reasons, further experiments evaluating nutrient requirements of Holstein × Gyr, including animals in different ages and gender, should be consider in an attempt to generate a database intending to elaborate a nutrient requirement system for dairy breeds used in tropical regions.

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Supporting information

S1 Spreadsheet. Comparison of estimantes of energy and protein requirements of crossbred Holstein x Gyr growing bulls with estimates from NRC (2000;2001) and BR-CORTE (2010). The spreadsheet included as supporting information can be used to meet energy and protein requirements for maintenance and growth using the equations adjusted in this study, the Nutrient Requirements of Dairy Cattle (NRC, 2001) and the BR-CORTE: Nutrient Requirements of Zebu Beef Cattle (Valadares Filho et al., 2010) in order to compare their estimates using diferente body weights and body weights gain. (XLSX)

CHAPTER 2

Interpretive Summary*:* Technical note: Assessment of the O_2P-HR method using respiration chambers and comparative slaughter for measuring the heat production of cattle. By Oss et al. Cattle expend energy as heat production in processes such as basal metabolism, digestive processes, voluntary activity and thermal regulation. Measurement of the heat production of cattle may enable determination of individual efficiency; the lower the heat production for the same weight gain the higher the efficiency of an animal. The scientific community interested in energy metabolism in cattle needs to have available techniques for measuring heat production and information about their capacity to make meaningful heat production measurements. Evaluating the reliability of methods for measuring heat production is important to scientists to be aware of the options that are suited to different experimental conditions.

Technical note: Assessment of the O_2P-HR method using respiration chambers and comparative slaughter for measuring the heat production of cattle

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ABSTRACT: The objective of this study was to assess the O_2P-HR (oxygen pulse (**O_2P**) and heart rate (**HR**)) technique using the respiration chamber (**RC**) and comparative slaughter (**CS**) methods for measuring the heat production (**HP**) of crossbred (Holstein×Gyr) yearling bulls. Twenty-four bulls were used. Six bulls were slaughtered at the beginning of the experiment as a reference group to estimate the initial empty body weight (**EBW**) and energy content of the remaining animals. The remaining bulls were assigned to a completely randomized design with 3 levels of DM intake, with 6 replicates. The levels of DM intake were 1.2% of BW, 1.8% of BW and *ad libitum*, with target orts of 5%. The bulls were fed a diet consisting of 59.6% corn silage and 40.4% concentrate on a DM basis. The HP (kcal/$BW^{0.75}$) was measured using three techniques, first using O_2P-HR followed by the RC and CS methods. The intercepts of the linear regressions (mean ± SE) were 21.08 ± 37.453 (H0: intercept = 0; P = 0.583) and 33.52 ± 29.917 (H0: intercept = 0; P = 0.280) for O_2P-HR *vs*. RC and O_2P-HR *vs*. CS respectively. The slopes of the linear regressions were 0.88 ± 0.229 (H_0: slope = 1; P = 0.620) and 0.76 ± 0.185 (H_0: slope = 1, P = 0.219) for O_2P-HR *vs*. RC and O_2P-HR *vs*. CS respectively. For both comparisons, the coefficients of determination (r^2), 0.52 and 0.53, and the concordance correlation coefficients (CCC), 0.70 and 0.73, were moderate for O_2P-HR *vs*. RC and O_2P-HR *vs*. CS respectively. The between-animal CV was higher for the O_2P-HR method (16.6%) compared to RC (7.7%) or CS (6.3%). We conclude that there was an agreement among the HP measurements detected using the assessed methods and that O_2P-HR may predict HP in cattle with high accuracy but moderate precision. Therefore, the O_2P-HR method may have limitations in terms of assessing HP in low numbers of replications due to higher between-animal CV than either the RC or CS methods.

Key words: heart rate, heat production, oxygen pulse

Technical note

The heat production (HP) of cattle may be determined under controlled and confined conditions by using the respiration chamber (RC) method; however, these conditions do not reflect those of free-ranging animals or of commercial cattle in feedlots or pastures. In an attempt to overcome the limitations of measuring HP in the cattle's environment, HP can also be measured using the comparative slaughter method (CS), as this technique allows the evaluation of animals that are being raised in several production conditions. However, the use of the CS method requires sacrificing animals and estimating DMI and metabolizable energy intake (**MEI**), which can increase labor and errors. The O_2P-HR method is an alternative technique for measuring HP that is based on long-term measurements (24 h periods) of the HR of free-range animals and on short-term measurements of O_2P (mL of O_2 consumed/heart beat), which are measured by attaching a face mask (**FM**) to the animal's nose (Brosh, 2007). Few experiments have been conducted to examine the reliability of the O_2P-HR method in measuring the HP of farm animals cows (Arieli et al., 2002; Brosh et al., 2002). However, to our knowledge, there are no studies comparing the O_2P-HR method with the more traditional techniques (RC and CS) of measuring HP mainly used in cattle. Therefore, the objective of this study was to compare the O_2P-HR, RC, and CS methods of measuring and assessing variation in HP in cattle. We hypothesized that the O_2P-HR method would predict HP measurements in a manner comparable to the RC and CS techniques.

The present study followed the Ethics Committee in Animal Use of the Universidade Federal de Viçosa (process number 44/2012). The experiment was conducted at the Multi-use

Complex on Livestock Bioefficiency and Sustainability at Embrapa Gado de Leite, in Coronel Pacheco, MG, Brazil, from August 2013 to February 2014.

Twenty-four Holstein×Gyr crossbred 10 months old bulls (initial body weight = 155.2 ± 5.6 kg) were randomly subdivided into 4 groups of 6 animals at the beginning of the experiment. One of those groups was designated as a baseline reference group and slaughtered at the beginning of the experiment to measure the initial body energy content in their EBW. The 3 remaining groups were fed at 3 levels of DM intake (1) 1.2% of BW, (2) 1.8% of BW or (3) *ad libitum*, target 5% orts. These treatments were chosen in order to achieve a variability in MEI and consequently variability in the results and the range of validity of the method. One animal from the *ad libitum* group had to be removed from the experiment due to health issues. Throughout the experiment, the bulls were housed in a tie stall system with free access to water and fed a diet consisting of corn silage and concentrate (59.6: 40.4 DM basis) once daily (08:30 AM). The concentrate was composed of soybean meal (24.8%), ground corn (67.9%), urea (2.4%), mineral mix (3.5%), and limestone (1.4%). The DM feed offered and refused was weighed to determine total daily DMI. The estimated metabolizable energy content of the diet was 2.4 Mcal/kg DM. The bulls were weighed at 15 d intervals. The animals from the 3 remaining groups were slaughtered at the end of the experiment to measure the final body energy content in their final EBW. The experiment lasted 173 d, 171 d, and 168 d for the 1.2% of BW, 1.8% of BW and *ad libitum* groups respectively, after which the animals were slaughtered. The slaughters followed the same procedures described by Costa e Silva et al. (2015).

The bulls were accustomed to the FM for a period of 2 wk prior to measurements. Following the training period, three O_2P (mL/heart beat) measurements were collected over a 3 d period, separated by 3 d of HR (beat/min) measurements at the tie stall.

The O_2 consumption data was recorded using a Sable System (Sable Systems International, Las Vegas, NV, USA) attached to the FM. Details about the gas measurements were described by Oss et al. (2016). Samples from the FM were collected at 20 s intervals and recorded at 1 min intervals over 20 min, with ambient air collected 5 min before and after the 20 min measurements to establish baseline gas levels. All data were recorded using an automated data acquisition program (Expedata, Sable Systems International, Las Vegas, NV, USA). The O_2 consumption (VO_2; mL/min) was calculated from the product of the STD flow rate (STDfr) and the difference in the average from the FM (O_2fm, % and baseline (O_2b; %), O_2 concentrations measurements over 30 min) as follows:

$$VO_2 = [\text{STDfr x } (O_2\text{fm} - O_2\text{b})]$$

The HR was recorded using a Polar equine transmitter and monitor (Model RS800CX, Polar Electro Inc., Kempele, Finland). The transmitters were embedded in a 10 cm wide girth strap with a velcro latch placed around the bulls' girth behind the shoulders. The negative electrode was positioned on the right side and the positive electrode on the opposite side of the bulls, parallel to the left elbow. The area around the electrodes was shaved and a conductivity gel was applied to increase conductance.

The HR measurements during the O_2 consumption measurement and the 3 days of HR at the tie stall were averaged and recorded every 60 sec. To obtain a representative daily average HR for each measurement day, a deviation was calculated for each 60 sec of HR recorded taking into account the average of 5 min before and 5 min after each minute recorded. Outliers were identified when this deviation was over 30%. The HR days were then overlapped and an average was calculated.

The O_2P (mL/heart beat) was determined by the average O_2 consumption per min over the average HR per min during the same 20 min period. Total daily O_2 consumption (L/d) was calculated from the average of O_2P and average daily HR. Daily HP was then calculated as the product of total daily O_2 consumption and the constant 20.47 KJ per liter of O_2 (McLean, 1972).

Two wk after performing the O_2P-HR method, the HP of the bulls was estimated using indirect open-circuit RC. Two RC were used. Bulls were housed in the chambers for two 24 h periods for HP measurement. Bulls were placed in the chamber, feed was delivered, the chamber was closed and measurements were initiated. After 24 h the measurements were interrupted and the bulls were removed to clean the chambers. The daily O_2 consumption and CO_2 and CH_4 production were measured over 24 h with correction for the CO_2 and CH_4 recovery levels in each chamber, which were 99.0% and 98.0% respectively. The HP was calculated based on Brouwer (1965).

With regard to CS, the HP was calculated as the difference between the MEI and retained energy (**RE**). The RE was calculated as the difference between the final energy content and the initial energy content in the EBW of bulls.

All HP were expressed as kcal/$BW^{0.75}$. The agreement between HP using O_2P-HR *vs*. RC and O_2P-HR *vs*. CS methods was analyzed using the Model Evaluation System (MES, Tedeschi, 2006; http://nutritionmodels.tamu.edu/models/mes/) in which predicted values (O_2P-HR) and observed (RC or CS) values were analyzed for accuracy and precision using several variables. These variables included linear regression of observed and predicted values, t-tests to identify the significance of parameters (intercept=0 and slope=1; Neter et al., 1996), coefficients of determination (r^2), concordance correlation coefficients (CCC; Lin, 1989), accuracy (Cb; Liao, 2003), the mean square error of prediction (MSEP), and its decomposition into mean bias,

systematic bias and random errors (Bibby and Toutenburg, 1977). The estimated means and standard deviations were used to compute between-animal coefficients of variation (CV) in HP (kcal/$BW^{0.75}$).

The relationships between HP (kcal/$BW^{0.75}$) measured using O_2P-HR *vs*. RC and O_2P-HR *vs*. CS methods are illustrated in Figure 1. Table 1 contains the description of the database of HP measurements for each method used. Related descriptive statistics are presented in Table 2.

For both comparisons, the regression analysis indicated that the slopes were not different from unity and the intercepts were not different from zero ($P > 0.050$), which is indicative of the high accuracy of the O_2P-HR method (Table 2). The intercepts of the linear regressions (mean ± SE) were 21.08 ± 37.453 (H0: intercept = 0; $P = 0.583$) and 33.52 ± 29.917 (H0: intercept = 0; $P = 0.280$) for O_2P-HR *vs*. RC and O_2P-HR *vs*. CS respectively (Table 2). The slopes of the linear regressions were 0.88 ± 0.229 (H0: slope = 1; $P = 0.620$) and 0.76 ± 0.185 (H0: slope = 1; $P = 0.219$) for O_2P-HR *vs*. RC and O_2P-HR *vs*. CS respectively (Table 2).

On the other hand, the regression estimates of r^2 were moderate, about 0.52 for the comparison with RC and 0.53 for the comparison with CS (Table 2). The r^2 is a good indicator of precision; the higher the r^2 the higher the precision (Tedeschi, 2006). Precision measures how close individual O_2P-HR values are within each condition (treatment), while accuracy measures how close O_2P-HR values are to the RC or CS values. Given these definitions of accuracy and precision, the O_2P-HR method has the ability to estimate the "correct" values because it showed high accuracy (Cb) compared to RC (0.97) and CS (0.99); however, it is not very sensible to measure values consistently when they present moderate precision (r^2). It is difficult to point out which is better, accuracy or precision. According to Tedeschi (2006) it is easy to understand why accuracy could be argued as the most important measure as the true mean can be detected using

an imprecise method simply by averaging a large number of data points. However, measurements using an accurate but imprecise method are unrealistic as they may interfere in the variation of means and consequently make it difficult to find differences between treatments using this technique.

Based on the discussion above, we believe that the O_2P-HR method has high accuracy and will provide good estimates of HP; however, to identify treatment differences in an experiment the sample size (*n*) must be increased. The O_2P-HR showed higher between-animal CV (16.6%) compared with RC (7.7%) and CS (6.3%). The implication of a higher between-animal CV is that to detect a treatment difference with a certain power, more animals (replicates) per treatment would be required with the O_2P-HR method compared to the RC or CS techniques.

The CCC, also known as the reproducibility index, indicates if the O_2P-HR measurements are precise and accurate at the same time. The closer to 1 the better. The CCC values were moderate, 0.70 for O_2P-HR *vs*. RC and 0.73 for O_2P-HR *vs*. CS, which was expected as the technique shows moderate precision. This analysis confirmed the high accuracy (Cb) of the adjustments, as the accuracy had to be higher than the r^2 in order to get a moderate CCC value.

The mean differences between observed (RC and CS) and predicted values (O_2P-HR) were 4.61 kcal/$BW^{0.75}$ for O_2P-HR *vs*. RC and -3.73 kcal/$BW^{0.75}$ for O_2P-HR *vs*. CS ($P < 0.001$; Table 1), indicating that O_2P-HR measurements were under-predicted relative to RC and over-predicted relative to CS. The MSEP calculation confirmed that most of the error associated with the O_2P-HR method was random error (98% compared to RC and 89% compared to CS. Table 2).

The O_2P-HR method generated HP measurements that were comparable, with high accuracy and moderate precision, to those estimated using RC and CS across a range of DM

intake levels. The O_2P-HR method showed a higher between-animal coefficient of variation, which has a negative impact on the power of an experiment using the O_2P-HR method. However, it is an alternative technique for those that do not have respiration chambers or who wish to avoid slaughtering animals and estimating DMI and MEI (needed to estimate HP by using CS), especially under grazing conditions. In general, this method may be a helpful tool for studies of energy metabolism in cattle but further studies should be performed to investigate ways to minimize errors associated with the *O_2P-HR* method in order to increase the precision and the statistical power of experiments using this technique.

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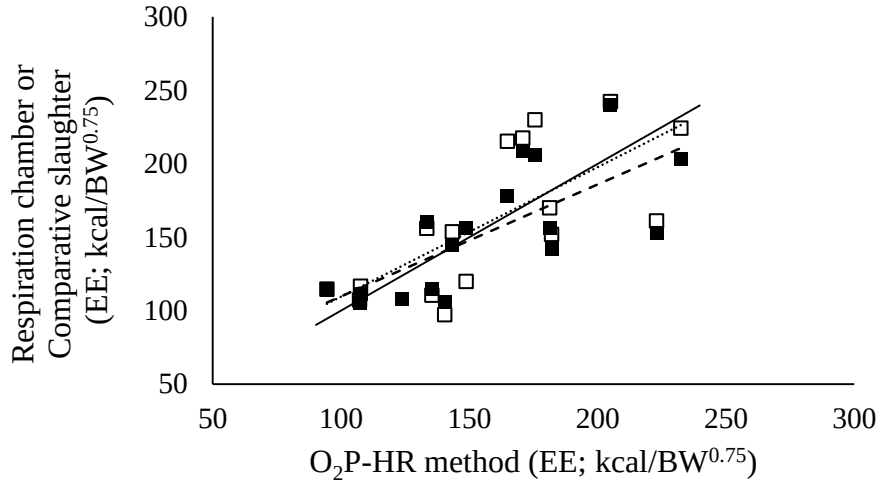

Figure 1. Relationships between heat production (HP; kcal/$BW^{0.75}$) measured using O_2P-HR *vs.* respiration chamber (RC) and O_2P-HR *vs.* comparative slaughter (CS) methods of individual crossbred (Holstein × Gyr) yearling bulls fed at different intake levels. Open symbols and dotted line are related to RC. Solid symbols and dashed line are related to CS. The solid line is the line $y = x$.

Table 1. Description of the database of heat production (HP; kcal/$BW^{0.75}$) measurements used to assess the O_2P-HR in comparison with respiration chamber (RC) and comparative slaughter (CS) methods

Method	Mean	Minimum	Maximum	SD	CV, %
O_2P-HR	157.1	94.6	232.5	40.03	16.6
RC	161.7	97.1	242.3	49.70	7.7
CS	153.3	105.3	239.8	41.89	6.3

Table 2. Descriptive statistic of relationship between heat production (HP; kcal/$BW^{0.75}$) using O_2P-HR *vs* respiration chamber (RC) and O_2P-HR *vs* comparative slaughter (CS) methods of individual crossbred (Holstein × Gyr) yearling bulls fed at different intake levels

Item	Methods comparison	
	O_2P-HR *vs* RC	O_2P-HR *vs* CS
Regression		
Intercept		
Estimate	21.08	33.52
Standard error	37.453	29.917
P-value[2]	0.583	0.280
Slope		
Estimate	0.88	0.76
Standard error	0.229	0.185
P-value[3]	0.620	0.219
r^2	0.52	0.53
CCC[1]	0.70	0.73
Cb[4]	0.97	0.99
MSEP[5]	1147.09	872.31
MB[6]	6.83	13.94
SB[7]	20.62	84.85
RE[8]	1119.64	773.52

[1]Correlation and concordance coefficient

[2]H0: intercept = 0

[3]H0: slope = 1

[4]Bias correction factor

[5]Mean square error of prediction

[6]Mean bias

[7]Systematic bias

[8]Random errors

CHAPTER 3

An evaluation of the face mask system based on short-term measurements compared with the sulfur hexafluoride (SF_6) tracer, and respiration chamber techniques for measuring CH_4 emissions

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ABSTRACT: The objective of the present study was to compare the short term measurement (30 min/d for 3 d) face mask system (FM), with SF_6 tracer and respiration chamber (RC) techniques for measuring CH_4 emissions from crossbred (Holstein × Gyr) yearling bulls fed at three intake levels. Data were derived from 17 individuals in a completely randomized design experiment in a repeated measures scheme. Bulls were fed a diet consisting of 59.6% corn silage and 40.4 % concentrate on a DM basis at three levels of DM intake (DMI) of 1.2% of BW, 1.8% of BW and *ad libitum*. After an adaptation period, CH_4 emissions were measured first using the SF_6 tracer technique, followed by the FM and RC techniques, respectively. Daily CH_4 emission (g/d) from bulls differed ($P<0.007$) with CH_4 measurements techniques, with highest emissions measured using RC (107.9 ± 15.36), followed by FM (103.2 ± 11.86) then SF_6 tracer technique (87.9 ± 10.16). The CH_4 emissions adjusted for differences in DMI and BW did not differ among techniques, averaging 21.5 g/kg DMI and 0.4 g/kg BW, respectively. Total CH_4 emissions (g/d) were positively correlated with DMI as measured by all three techniques (SF_6 r = 0.93; FM r = 0.93; RC r = 0.96). The concordance correlation coefficient (CCC) for CH_4 emission (g/d) were 0.82, 0.82 and 0.74 for comparisons of SF_6 *vs* RC, FM *vs* RC and FM *vs* SF_6, respectively. The day-to-day (21.3%) and animal-to-animal (13.4%) variation in CH_4 yield (g/kg DMI) was greater from bulls using the FM technique, compared to SF_6 (18.8% and 6.8%, respectively) and RC (12.9% and 7.5%, respectively) techniques. We conclude that the short-term FM technique generated CH_4 measurements that were comparable to those estimated using SF_6 and chamber techniques across a range of DMI levels. However, the FM method may have limitations in terms of assessing enteric CH_4 mitigation strategies that are applied over a short duration to low numbers of animals due to higher animal-to-animal and day-to-day coefficients of variation than either the SF_6 or RC techniques.

Keywords: face mask; methane; respiration chamber; short-term measurement; sulfur hexafluoride

Introduction

Whole animal open-circuit indirect-respiration calorimetry is often mentioned as the "gold standard" because it has been proven to be accurate in CH_4 measurements, with a low coefficient of variation (Blaxter and Clapperton, 1965; Grainger et al., 2007). However, the capital investment for RC is high, animals must be trained and the behavior of the individual may be altered from that which occurs under most production settings e.g. reductions in DMI (Johnson and Johnson, 1995). In an attempt to overcome these constraints, the SF_6 tracer technique was developed (Johnson et al., 1994) which eliminates the need for confinement of the animal, allowing for CH_4 emissions to be estimated under grazing conditions. However, compared to RC , the SF_6 tracer technique may underestimate (McGinn et al., 2006) CH_4 emissions in some instances and overestimate (Muñoz et al., 2012) it in others. More importantly, the day-to-day and animal-to-animal variation in CH_4 emission estimates may be greater with the SF_6 tracer technique as compared to estimates derived from chambers (Pinares-Patino et al., 2011; Munoz et al., 2012).

Currently, alternative methods to estimate enteric CH_4 emissions are based on spot sampling and include the GreenFeed system (GF; C-Lock Inc., Rapid City, South Dakota, USA; Hammond et al., 2015), portable gas quantification system (GQS; Dorich et al., 2015; Huhtanen et al., 2015), portable static chambers (PSC; Goopy et al., 2011) and many others.

The FM technique has also been cited as an alternative system to measure CH_4 emission from ruminants (Johnson and Johnson, 1995; Bhatta et al., 2007). The principle of this technique is similar to RC in terms of measuring gas exchange and changes in exhaled CH_4 concentration.

In the past, the FM was performed for short-term measurements (i.e. 30 min) taken every 2-3 h with up to 7 measurements per day (Washburn and Brody, 1937; Kawashima, 2001). This number of measurements presented a marked impact on animal behaviour, as access to food and water was restricted during measurement periods. Consequently, the FM technique was considered too laborious and interest in using the method to measure enteric CH_4 from ruminants faded.

Daily CH_4 emission profiles of ruminants usually exhibit a diurnal pattern in relation to the feeding time and level of feed consumption (Crompton et al., 2010; *Hünerberg* et al., 2015). The diurnal pattern associated with feed consumption exhibits a continuous rise to a peak followed by a period of reasonably linear decline. However, it has been demonstrated that short-term measures of CH_4 emission may be strongly correlated with daily CH_4 emission depending on the time after feeding at which the short-term measurement is made (Goopy et al., 2011; Pickering et al., 2015; Robinson et al., 2015).

We believe that it would be possible to reduce the number of measurements per day using the FM system if sampling is conducted at a time that strongly correlates with total daily CH_4 emission. The short-term FM technique has advantages as it is comparatively cheaper and simpler compared to other techniques such as SF_6 or RC. Its mobility allows it to be moved to multiple housing sites to collect CH_4 emission estimates, and it has also been used as a method to measure energy expenditure and energy balance of ruminants (Brosh, 2007).

The objective of this study was to compare the FM (based on short-term measurements), SF_6 tracer and RC techniques for measuring and assessing variation in CH_4 emissions in yearling crossbred bulls (Holstein × Gyr) fed at three different intake levels. It was hypothesized that the

FM method would predict daily CH_4 measurements in a manner that is comparable to the SF_6 and RC techniques.

Materials and methods

The present study followed the Ethics Committee in Animal Use of the Universidade Federal de Viçosa (process number 44/2012). The experiment was conducted at the Multi-use Complex on Livestock Bioefficiency and Sustainability at Embrapa Dairy Cattle, in Coronel Pacheco, MG, Brazil, from August 2013 to February 2014. The CH_4 emissions were estimated between October and December 2013.

Animals, Diet and Experimental Design

Eighteen Holstein × Gyr crossbred 10 months old bulls (initial live weight = 155.2 ± 5.6 kg) were randomly subdivided into 3 groups of 6 animals at the beginning of the experiment. Treatments consisted of three intake levels (IL): (1) 1.2% of BW; (2) 1.8% of BW and (3) *ad libitum* - target 5% orts. One animal from the *ad libitum* group had to be removed from the experiment due to health issues. Throughout the study bulls were housed in a tie stall system with free access to water, and were fed a diet consisting of corn silage and concentrate (59.6: 40.4 DM basis) once daily (0830 h). Feed DM offered and refused were weighed to determine total daily DMI. Body weight was measured at 15 d intervals. Dry matter intake was estimated in the interim period between each measurement method, and the amount of feed offered to each bull was adjusted accordingly.

The concentrate was composed of: soybean meal (24.8%), ground corn (67.9%), urea (2.4%), mineral mix (3.5%) and limestone (1.4%). The diet had a DM content of 417 g/kg, and

CP, NDF and non-fiber carbohydrates (NFC) contents of 148, 336 and 426 g/kg DM, respectively. The estimated metabolizable energy content of the diet was 2.4 Mcal/kg DM.

Ingredients of the concentrate were collected for analysis and representative samples of silage, concentrate and orts were collected daily, and pooled monthly for chemical analysis. Samples were analysed using Association of Official Analytical Chemists (AOAC 1995) methods for analytical DM (method 930.15) and CP (method 990.03). Gross energy was determined using an adiabatic calorimeter (model C-5000, Labcontrol IKA, São Paulo, SP). Neutral detergent fiber was determined according to Van Soest et al. (1991) with a heat stable amylase and expressed exclusive of residual ash.

Bulls were adapted to the diets and measurement technique equipment for 5 wk prior to the start of CH_4 measurements. During this 5 wk period, bulls were trained to become accustomed to the SF_6, FM and RC techniques. In the second week of the 5 wk period, bulls were adapted to RC by placing them in the chambers prior to the morning feeding and returned to the tie stall at 1600 h for 3 d. The adaptation to the SF_6 method occurred after all bulls were adapted to RC. The canisters for SF_6 measurements were placed on each bull for 1 wk. Bulls were accustomed to the FM for a period of 2 wk prior to measurements. Bulls were placed in a squeeze chute and the face mask was fitted for two 20 min periods at about 1000 h and 1400 h. The techniques were evaluated in the following order: SF_6 was performed for 5 consecutive days of CH_4 measurements, FM was performed for 30 minutes of CH_4 measurements/d for 3 d and RC was perfomed for 2 periods of 24 h CH_4 measurements.

SF_6 tracer measurements

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 8 wk within a controlled environment at 39^{o}C. The release rates of SF_6 tubes ranged from 1.19 to 2.31 mg/d, with a mean of 1.86 ± 0.314 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 bull 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 was collected by placing a head collar on each bull 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 bulls, gas was sampled and the pressure was recorded. Background canisters were collected for analysis at the same time as canisters were collected from bulls. 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 seperate 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 300°C and N_2 at 40 mL/min was used as the carrier gas. Oven temperature was kept at 50°C for 4 min to elute the desired constituents. The gas chromatograph was calibrated weekly using SF_6 (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 280°C, 10 mL/min of H_2 flow, 400 mL/min of synthetic air, 20 mL/min of complementation flow. Oven temperature was kept at 50°C 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):

$$ECH_4 = RSF_6 \frac{[CH_4]M - [CH_4]BG}{[SF_6]M - [SF_6]BG} \times \left(\frac{MW\ CH_4}{MW\ SF_6}\right) \times 1000$$

Face Mask measurements

Following the SF_6 technique, cattle were adapted for 2 wk to the FM technique and a single 30-min CH_4 measurement was performed each day for 3 d by placing bulls in a squeeze chute and collecting measurements 6 h (1430h) after feeding at 0830 h. This duration after feeding was selected as it has been proposed to be highly correlated with average daily CH_4 emissions (Grainger et al., 2007; Crompton et al., 2010). A total of four bulls were evaluated each day. Their diet was delivered at planned intervals so as to achieve the measurements at about 6 hours after feeding**.** The CH_4 emission data was recorded with the Sable System (Sable Systems International, Las Vegas, NV, USA) attached to each 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). 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. 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 sub-sampling pump (SS-4 Sub-Sampler Pump) at 200 mL/min.

Samples from the FM were collected at 1 min intervals over 20 min, with ambient air collected over 5 min before and after the 20 min measurement to establish baseline gas levels. Humidity levels of the samples were monitored by a humidity meter (RH-300; Sable Systems International, Las Vegas, NV, USA), prior to flowing through a CH_4 infrared gas analyser (MA-10, Sable Systems, Henderson, NV). All data were recorded with an automated data acquisition

program (Expedata; Sable Systems International, Las Vegas, NV, USA). 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 STD flow rate (STDfr) and the difference in the average of the FM (CH_4fm; % and baseline (CH_4b; %); CH_4 concentrations measurements over 30 min) as follows:

$$VCH_4 = [\text{STDfr x } (CH_4\text{fm} - CH_4\text{b})]$$

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 g CH_4 = 1.4 L CH_4).

Chamber design, operation, and CH_4 measurements

Four wk after FM measurements, CH_4 emissions from bulls was estimated using indirect open-circuit RC. Two RC were used with a net volume of 21.1 m^3 (3.68 m long, 2.56 m wide and 2.24 m high). The ambient 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 analysis of 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 CH_4 concentrations, flow rate, temperature, barometric pressure and water vapour pressure during the measurement period. Estimates were corrected for differences in water vapour, lag time and drift, with CH_4 emission (L/min) being calculated for each chamber at 15 min intervals. One day before placement in the chambers for CH_4 measurement, bulls were accoustomed to two extra chambers for 24 h. Bulls were housed in the chambers for two 24 h periods of CH_4 measurement. Bulls were placed in the chamber, diet was delivered, the chamber was closed and measurements were initiated immediately. After 24 h, measurements were interrupted and bulls were removed for cleaning of the chambers. Another 24-h period was performed as described above. Bulls were weighed at the start and end of the measurement period. The CH_4 emissions were estimated over 24 h with correction for the recovery level in each chamber.

Statistical Methods

Outliers were identified and excluded from the dataset when the studentized residual was outside the range of -2.5 to 2.5 (Neter et al., 1996). Data were analyzed as a completely randomized design in a repeated measures scheme using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model included the fixed effect of measurement technique and intake level, and the random effects of animal within intake level. Significance was declared at $P \leq 0.05$. Lin's concordance coefficients were generated to enable comparison among CH_4 emissions (g/d) from the assessed techniques (Lin, 1989; Lin, 1992). The correlation coefficient was used to calculate the concordance correlation coefficients (CCC; Hamlett et al., 2004). The correlation coefficient was used to determine precision over the range of CH_4 emissions by determining the deviation of the data from the best-fit line. The CCC was used to determine accuracy by determining how much the best-fit line deviated from the line $y = x$. The estimated means and standard deviation, excluding outliers from data, were used to compute day-to-day and animal-to-animal coefficients of variation (CV) for CH_4 yield (g/kg DMI). Based on the animal-to-animal CV detected for FM technique, a power analysis was performed to determine the sample size required in a experiment using the FM method (Ryan, 2013).

Results

Values of CH_4 obtained using SF_6 tracer, FM and RC techniques

Body weight and DMI differed among techniques (P<.001; Table 1). Taking into account all intake levels for each technique, average CH_4 emissions (g/d) were 87.9 ± 10.16 g/d (mean ± SEM) for the SF_6 tracer technique, 103.2 ± 11.86 g/d for the FM, and 107.9 ± 15.36 g/d for RC (Table 1). For CH_4 emission (g/d) the interaction between intake level (IL) and technique (Tech) was significant (P=0.006; Table 1). Greater CH_4 emission (g/d) was associated with the *ad libitum* group with RC as compared to other measurement techniques, and a greater CH_4

emission (g/d) at feeding levels of 1.8% of BW was found with the FM when compared to SF_6 technique. When CH_4 emissions (g/d) were adjusted for differences in DMI and BW (g/kg DMI or g/kg BW), no differences were observed (P>0.05) among measurement techniques (Table 1). Irrespective of measurement technique, CH_4 emissions (g/d) increased (P<0.001) with increasing DMI.

Correlation and concordance analysis

Correlation coefficients (*r*) between CH_4 emission (g/d) and DMI were 0.93 (P<0.001), 0.93 (P<0.001) and 0.96 (P<0.001) for the SF_6 tracer, FM and RC techniques, respectively (data not shown). Correlations between CH_4 emission (g/d) and BW were lower at 0.83 (P<0.001) for SF_6, 0.89 (P<0.001) for FM, and 0.93 (P<0.001) for the RC method (data not shown). Correlations between CH_4 emissions (g/d) measured using the SF_6 (0.94; P<0.001) or FM (0.85; P<0.001) techniques with RC measurements was high (Figure 1*)*. The CCC for CH_4 emission (g/d) using SF_6 vs RC, FM vs RC and FM vs SF_6 were 0.82, 0.82 and 0.74 respectively (Figure 1). The location shift (intercept) indicates how the y intercept of the plotted data differs from the y intercept of the line y = x (the line y = x would have a location shift of zero). These shifts with regard to CH_4 emission (g/d) of the SF_6 vs RC and FM vs RC were negative whereas the intercept for FM vs SF_6 techniques was positive *(Figure 1).* The negative intercepts of the relationship CH_4 emission (g/d) comparing the SF_6 vs RC and FM vs RC indicate that the SF_6 and the FM techniques underestimated CH_4 emissions as compared to RC. In contrast, the positive intercept for FM and SF_6 techniques suggests that FM overestimated CH_4 emissions relative to SF_6 technique. The scale shift (slope) indicates the discrepancy in slope between the plotted data and the line y = x (slope of a 1:1 line would equal 1.0). For SF_6 vs RC, FM vs RC

and FM vs SF_6, the scale shifts (slope) were 1.4, 1.1 and 0.68 respectively (Figure 1). These scale shifts indicate a moderate discrepancy in slope between the plotted data and the line y = x.

Variability in CH_4 yield using SF_6 tracer, FM and RC techniques

The FM technique had greater average day-to-day (21.3%) and animal-to-animal variation (13.4%), in comparison to SF_6 and RC techniques (Table 2). The SF_6 tracer technique averaged greater day-to-day (18.8%) and lower animal-to-animal (6.8%) variations compared to the RC technique (12.9% and 7.5%, respectively; Table 2). The power analysis demonstrated that the FM technique with a greater animal-to-animal CV, when compared to the SF_6 and RC techniques, would require more animals (replicates) per treatment to detect a 5%, 10% or 15% treatment difference with 80% of power (Table 3).

Discussion

The interest in CH_4 techniques based on spot sampling is increasing due to its simplicity and propensity to increase the number of animals measured. Our objective was to compare a short-term measurement using the FM system to long-term measurements of CH_4 emissions from cattle using the SF_6 and RC techniques. To our knowledge, there are no studies comparing CH_4 values obtained using FM system with values obtained using SF_6 and RC techniques. Our hypothesis was that the FM method would accurately predict daily CH_4 values compared to SF_6 tracer and RC, based on samples for measurement using the FM collected at a fixed time after feeding.

Comparison of CH_4 values using different techniques by overall means

The variability in CH_4 between techniques is due to several factors – most commonly with the technique itself and its execution. The measurement errors associated to the SF_6 tracer technique have led to the development and validation of a modified SF_6 method, by replacing capillary tube flow restrictores with orifice plate flow controllers in the sampling apparatus which was found to be concordant with RC technique (Deighton et al., 2014).

In our study, CH_4 emission (g/d) from bulls using SF_6 and FM were found to be 18% and 4% lower than those measured using RC, respectively. Usually, in studies of comparison of methods for measuring CH_4 emissions, differences in CH_4 emissions (g/d) between the original SF_6 tracer and the RC techniques are in part attributed to the flatus emission that would not be measured by the SF_6 procedure (Muñoz et al., 2012). The FM method also fails to account for flatus emissions. However, CH_4 from flatus emission makes a very small contribution (2%) to the total daily CH_4 emission as about 90% of the CH_4 originates in the rumen and 10% in the hindgut. As 90% of the CH_4 produced in the intestine is absorbed into the blood and released by respiration, emissions through the rectum are low (Murray et al., 1976). Undoubtedly, some of the lower CH_4 emissions (g/d) associated with the SF_6 and the FM technique in our study reflect the lower DMI of the bulls during these measurement periods as compared to the time that RC measurements were made.

Differences in CH_4 emissions (g/d) among different intake levels were expected as CH_4 emission by ruminants is primarily determined by DMI, which determines the level of substrate available for fermentation in the rumen. The CH_4 emissions are positively correlated with DMI (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995) which was also supported by this study. When CH_4 was expressed as CH_4 yield (g/kg DMI) there was no differences in emissions

as assessed by the three techniques indicating agreement among techniques. In our study, CH_4 yield averaged 21.5 g/kg DMI.

Comparison of CH_4 emission using different techniques by correlation and concordance test

As the assessed techniques were not perfomed simultaneously, Lin's (1989) concordance correlation coefficient analysis was used to test agreement among techniques in an attempt to overcome the limitation of comparing CH_4 emissions (g/d). In previous work, Lin's concordance correlation coefficients have been use to test for continuity in CH_4 emissions (g/d) estimated using SF_6 *vs* RC techniques (McGinn et al., 2006) and between the GF *vs* RC and GF *vs* SF_6 techniques (Hammond et al., 2015).

Although average CH_4 emission (g/d) was different among techniques, the high correlations found between CH_4 emissions (g/d) measured using the SF_6 or FM techniques with RC measurements indicate agreement among the techniques assessed. This concordant result may suggest the possibility of using the FM, based on short-term measurements, to estimate CH_4 emission from cattle.

Variability in CH_4 yield using different techniques

Several studies have also shown that the variation in CH_4 yield measured using the SF_6 technique was generally greater than the variation in CH_4 yield measured using the RC (Vlaming et al., 2008; Hammond et al., 2009, 2015). However, research using the 'modified' SF_6 technique reported an animal-to-animal CV in daily CH_4 yield of 6.5%, compared to 7.5% using RC (Deighton et al., 2014).This is similar to our animal-to-animal CV for CH_4 yield using SF_6 (6.8%) and RC (7.5%).

With regard to techniques for measuring CH_4 emissions from ruminants based on short-term measurements, their accuracy and precision are still poorly defined (Cottle et al., 2015). The FM technique presented a greater day-to-day (21.3%) and animal-to-animal CV (13.4%) compared to SF_6 and RC techniques (Table 2). It was previously reported that short-term CH_4 emission measurements present greater animal-to-animal and day-to-day variation compared to RC (Robinson et al., 2012; Zimmerman et al., 2013).

Low animal-to-animal CV can increase the statistical power of experiments that seek to measure CH_4 emissions. The implication of an animal-to-animal CV of 13.4% is that to detect a 10% treatment difference with 80% of power, 20 more animals (replicates) per treatment would be required with the FM as compared to the SF_6 or RC techniques (Table 3). Therefore, despite the FM technique being simpler and less expensive, it is recommended only in situations where more animals are availabe for experimentation.

Implications of using the FM system for measuring CH_4 emission

The development of simple and low-cost techniques for measuring enteric CH_4 production is one of the areas that should have high priority for use in the assessment of future CH_4 mitigation strategies and for inventory purposes. We understand that the applicability of a method depends on different resources and availability of technicians. *T*he success of using short-term emission techniques is to ensure that sampling times are consistently undertaken in relation to the time of feed consumption. Therefore, the feed management of animals in an experiment using FM to measure CH_4 should be adjusted to coincide with time of feeding in order to generate meaningful CH_4 emission estimates (Hegarty, 2013). Ensuring that measurements were consistently conducted 6 h after feeding once a day, likely enabled the FM

procedure to generate representative daily CH_4 emission estimates for animals fed once daily and with intake up to 9 kg. In our study, short-term measurements using the FM method (*30 min/d for 3 d,* 6 h after feeding) did generate CH_4 estimates that were comparable to those obtained with SF_6 and RC techniques. Performing one measurement per day could decrease the duration of stress that animals are subjected to, with minimal alteration in water and feed intake. Further investigations should be performed to investigate other ways to minimize errors associated with the FM technique in attempt to increase the statistical power of experiments using this technique. Other challenge on the use of the FM method is related to its application with grazing animals where the forage intake follow a different pattern from confined animals, and it may influence on the precision and accuracy of the CH_4 measurements, which should reflect a measurement of an entire day; once the success of a CH_4 technique based on short-term measurements is dependent of when the CH_4 measurement is performed related to the time of feed consumption. We do not have knowledge about the pattern of the CH_4 emission in grazing animals and therefore, when would be the best time to perform the short-term measurement.

Conclusions

We conclude that there was a general agreement among methane measurement techniques of SF_6, FM and RC for the determination of enteric CH_4 emissions from bulls. The FM technique presented a greater day-to-day and animal-to-animal variation compared with SF_6 and RC methods. This greater variation has a negative impact on power of an experiment using the FM, and it would require more animals (replicates) to detect treatment difference with 80% of power. However, it is a low-cost and simple alternative to estimate CH_4 emissions compared to the more conventional techniques for inventory purposes and for evaluating the effects of mitigation strategies, besides to facilitate screening of more animals. We suggest further

evaluation of the FM technique to determine how best to deploy the system to meet specific objectives.

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Table 1. Comparison of CH_4 emissions measured using the sulfur hexafluoride (SF_6) tracer, face mask (FM) and respiration chamber (RC) techniques (Tech) when crossbred young bulls (Holstein × Gyr) had different intake levels (IL)

Item	1.2% of BW[1]			1.8% of BW[2]			*Ad libitum*[3]			SEM	P-value		
	SF_6	FM	RC	SF_6	FM	RC	SF_6	FM	RC		IL	Tech	IL × Tech
BW (kg)	174.3^{b}	189.9^{a}	188.1^{a}	201.6^{c}	229.9^{b}	258.9^{a}	242.7^{c}	286.2^{b}	346.5^{a}	21.79	0.001	<.001	<.001
DMI (kg)	2.04^{a}	2.35^{a}	2.33^{a}	3.63^{c}	4.28^{b}	4.79^{a}	7.22^{b}	7.10^{b}	8.56^{a}	0.522	<.001	<.001	0.001
CH_4 (g/d)	50.4^{a}	55.4^{a}	48.5^{a}	75.3^{ac}	101.8^{ab}	87.4^{a}	138.1^{b}	152.5^{b}	187.9^{a}	14.37	<.001	0.007	0.006
CH_4/BW[4] (g/kg)	0.29	0.30	0.26	0.38	0.44	0.34	0.56	0.53	0.54	0.456	<.001	0.255	0.395
CH_4/DMI[5] (g/kg)	24.7	23.8	20.9	21.0	23.6	18.3	19.0	21.4	20.9	2.54	0.112	0.238	0.239

[1]DM supply restricted to 1.2% of BW

[2]DM supply restricted to 1.8% of BW

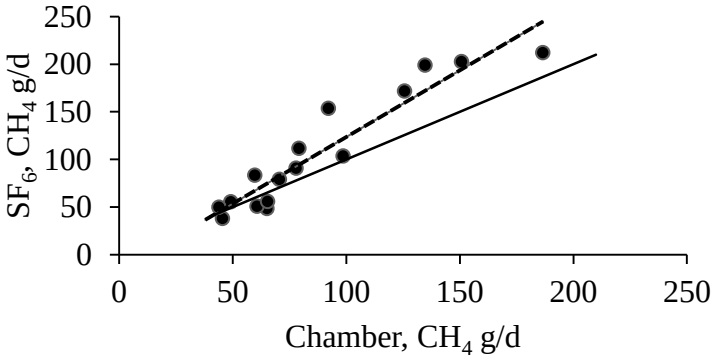
[3]*Ad libitum*; Target 5% orts

[4]Daily CH4 emissions (g/d) per unit of BW

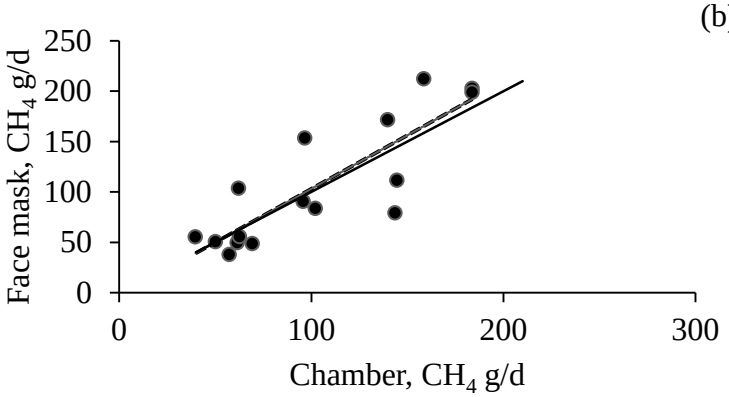
[5]Daily CH4 emissions (g/d) per unit of DMI

a-c Means within intake level in a row followed by different letters differ (P≤0.05)

(a)


250
200
150
100
50
0
SF6, CH4 g/d
0
50
100
150
200
250
Chamber, CH4 g/d

(b)


250
200
150
100
50
0
Face mask, CH4 g/d
0
100
200
300
Chamber, CH4 g/d

(c)

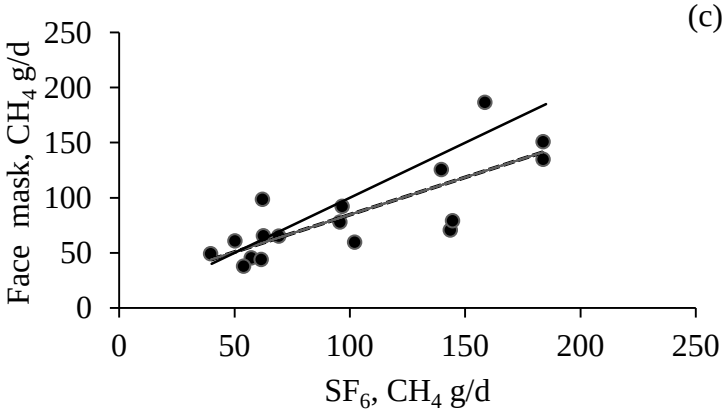

250
200
150
100
50
0
Face mask, CH4 g/d
0
50
100
150
200
250
SF6, CH4 g/d

Figure 1. Relationships between CH_4 emission (g/d) determined using SF_6 *vs* respiration chamber (RC) techniques (a: y = 1.4016x – 16.573; R^2 = 0.8927; P<.0001), face mask (FM) *vs* RC techniques (b: y = 1.0625x – 3.047; R^2 = 0.7178; P<.0001) and FM *vs* SF_6 techniques (c, y = 0.6779x + 16.942; R^2 = 0.6263; P=0.0002), of individual yearling bulls fed at different intake levels. Solid line indicates y = x. The correlation coefficients were 0.9448, 0.8472 and 0.7914 for figure 1a, 1b and 1c respectively. Lin's concordance value (CCC) for figure 1a, 1b and 1c were 0.8232, 0.8244 and 0.7398, respectively.

Table 2. Day-to-day and animal-to-animal coefficients of variation of CH_4 yield (g/kg DMI) as measured using SF_6 tracer, face mask (FM) and respiration chamber (RC) techniques when crossbred yearling bulls (Holstein × Gyr) had different intake levels (IL)

IL	Techniques		
	SF_6	FM	RC
	Day-to-day variation		
1.2%[1]	17.1	25.8	14.2
1.8%[2]	16.6	25.8	10.9
AdL[3]	22.8	12.4	13.6
Average	18.8	21.3	12.9
	Animal-to-animal variation		
1.2%	6.7	24.6	12.5
1.8%	7.4	7.7	4.0
AdL	6.2	7.9	6.0
Average	6.8	13.4	7.5

[1]DM supply restricted to 1.2% of BW

[2]DM supply restricted to 1.8% of BW

[3]*ad libitum*; Target 5% orts

Table 3. The number of replicates (animals) required to detect as significant at 5% of level a 5%, 10% or 15% two treatment difference in CH_4 yield (g/kg DMI), with a probability of detector (power) of 80% using a two-tailed, two-sample *t*-test, given averaged between-animal CV found for SF_6, face mask (FM) and respiration chamber (RC).

Treatment difference	Techniques		
	SF_6[1]	FM[2]	RC[3]
5%	31	114	37
10%	9	30	10
15%	5	26	6

[1]Animal-to-animal CV: 6.8%

[2]Animal-to-animal CV: 13.4%

[3]Animal-to-animal CV: 7.5%