Universidade Federal de Minas Gerais

Eficiência alimentar e características produtivas de bezerras Gir em aleitamento

Camila Flávia de Assis Lage

Belo Horizonte

2019

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Tese apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para obtenção do grau de Doutor em Zootecnia.

Área de concentração: Produção Animal

Prof. Orientador: Sandra Gesteira Coelho
Prof. Coorientador: Mariana Magalhães Campos e Fernanda Samarini Machado

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ESCOLA DE VETERINÁRIA DA UFMG
COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA
Av. Antônio Carlos 6627 - CP 567 - CEP 30123-970 - Belo Horizonte- MG
TELEFONE (31)-3409-2173

www.vet.ufmg.br/academicos/pos-graduacao
E-mail cpgzootec@vet.ufmg.br

ATA DE DEFESA DE TESE DE CAMILA FLÁVIA DE ASSIS LAGE

Às 08:00min horas do dia 07 de outubro de 2019, reuniu-se, na Escola de Veterinária da UFMG a Comissão Examinadora de Tese, indicada pelo Colegiado em reunião no dia 09/09/2019, para julgar, em exame final, a defesa da tese intitulada: EFICIÊNCIA ALIMENTAR E CARACTERÍSTICAS PRODUTIVAS DE BEZERRAS GIR EM ALOJAMENTO, como requisito final para a obtenção do Grau de **Doutor em Zootecnia área de concentração Produção Animal.**

Abrindo a sessão, o Presidente da Comissão, Profa. Sandra Gesteira Coelho, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Tese, passou a palavra ao (a) candidato (a), para apresentação de seu trabalho. Seguiu-se a argüição pelos examinadores, com a respectiva defesa do candidato (a). Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento da tese, tendo sido atribuídas as seguintes indicações:

	Aprovada	Reprovada
Prof.(a)/Dr.(a) Thierry Ribeiro Tomich	X	
Prof.(a)/Dr.(a) Ricardo Reis e Silva	X	
Prof.(a)/Dr.(a) Rafael Alves de Azevedo	X	
Prof.(a)/Dr.(a) Helton Mattana Saturnino	X	
Prof.(a)/Dr.(a) SANDRA GESTEIRA COELHO	X	

Pelas indicações, o (a) candidato (a) foi considerado (a):

- [X] Aprovado (a)
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Para concluir o Doutorado, o(a) candidato(a) deverá entregar 3 volumes encadernados da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente ao (a) candidato (a) pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

Belo Horizonte, 07 de outubro de 2019.

Assinatura dos membros da banca:

(Vide Normas Regulamentares da defesa de Tese no verso)
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Para um caminho que você escolhe, existe outros tantos que você abandonou...Talvez, aprender a conviver com isso e apreciar cada parte do caminho e do plantio seja a melhor forma de aceitar a colheita final. Quase 11 anos se passaram desde que eu decidi ser Médica Veterinária. Muitas coisas e pessoas me influenciaram a tomar as decisões que me guiaram até aqui, com alegria ou com dor. Muito provavelmente frustrei as expectativas de muitas pessoas, assim como tive muitas frustrações pelo caminho ... Não trocaria ou mudaria minha jornada, porque ela é única, e minha.

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Jorge Luiz de Alencar Soares

“Um navio está seguro no porto, mas não e pra isso que os navios foram feitos”
Willian Shedd

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AE – Alta eficiência

AGV – Ácidos graxos voláteis

BE – Baixa eficiência

BHBA – Beta hidróxido butirato

CAR – Consumo alimentar residual

CGR – Consumo e ganho de peso residual

CMS – Consumo de matéria seca

DS – Dieta sólida

EA – Eficiência alimentar

EB – Energia bruta

ECC – Escore de condição corporal

ED – Energia digestível

EL – Energia líquida

EM – Energia metabolizável

GMD – Ganho médio diário

GPR – Ganho de peso residual

MO – Matéria orgânica

PC – Produção de calor

PDR – Proteína degradável no rúmen

PM – Proteína microbiana

PV – Peso vivo

TGI – Trato gastrointestinal

TIV – Termografia infravermelha

ACRONYMS AND ABBREVIATIONS LIST

AA - Amino acids

ADG – Average daily gain

BHB - Beta hydroxybutyrate

BW – Body weight

$BW^{0.75}$ – Metabolic weight

CH4 - Methane

CO2 – Carbon dioxide

CoA – Coenzime A

CP – Crude protein

DEI – Digestible energy intake

DM – Dry matter

DMI – Dry matter intake

EB – Energy balance

EE – Ether extract

FE – Feed efficiency

GE – Gross energy

GEI – Gross energy intake

HE – High efficiency

HP – Heat production

IRT – Infrared thermography

LE – Low efficiency

MEI – Metabolizable energy intake

NDF – Neutral detergent fiber

NE – Net energy

$N\text{-}NH_3$ – Amoniacal nitrogen

O_2 – Oxigen

OM – Organic matter

PETG - Polyethylene terephthalate glycol

RFI – Residual feed intake

RG – Residual gain

RH – Room humity

RIG – Residual intake and body weight gain

SD – Standard deviation

SEM – Standard error of means

TSD – Total solid diet

VCH_4 – Methane volume

VCO_2 – Carbon dioxide volume

VFA – Volatile fatty acids

VO_2 – Oxygen volume

RESUMO

Os objetivos deste estudo foram: 1) classificar os animais em grupos de alta e baixa eficiência utilizando dois índices de eficiência alimentar (consumo alimentar residual (**CAR**) e consumo e ganho de peso residual (**CGR**), e 2) avaliar se bezerras durante a fase de aleitamento, divergentes para índices de eficiência alimentar, apresentam diferenças em desempenho, medidas corporais, parâmetros sanguíneos e ruminais, digestibilidade, partição de energia, partição de nitrogênio e termografia. Trinta e duas bezerras Gir foram utilizadas no experimento com 63 dias de duração e classificadas em dois grupos de eficiência alimentar (**EA**) baseados no CAR e CGR (média ± 0,5 DP). Os grupos foram classificados como CAR de alta eficiência (**AE**) (AE CAR, n = 9; AE CGR, n = 10) e CAR de baixa eficiência (**BE**) (BE CAR, n = 10; BE CGR, n = 11). As bezerras de AE e BE apresentaram valores de CAR de - 0,052 e 0,049 kg / d ($P < 0,05$), respectivamente). Ao nascimento, a quantidade de leite integral fornecida para cada animal foi calculada com base no seu peso metabólico ao nascimento (42% x $PV^{0,75}$). A dieta líquida foi dividida em duas refeições às 07:00 e 14:00 horas. A dieta sólida total (**DS**) foi composta de 92% de concentrado e 8% de feno de tifton 85 picado em 5 cm de comprimento. A ingestão foi medida diariamente. O peso corporal e as medidas foram realizadas no nascimento, terceiro e sétimo dia e semanalmente em diante. As concentrações sanguíneas de insulina, BHB, ureia e glicose, além de pH, N-NH_3 e ácidos graxos voláteis (**AGV**) no líquido ruminal foram avaliadas aos 14, 28, 42, 56 e 70 dias de idade, respectivamente. Do 50º ao 55º dia de idade, os animais foram submetidos a um ensaio de digestibilidade. Aos 55 (± 6) dias de idade, as trocas respiratórias foram mensuradas por uma câmara respirométrica de circuito aberto. As imagens térmicas dos bezerros foram realizadas com uma câmera de infravermelho em d 56 (± 3) às 06:00 h, antes da alimentação. O grupo AE CAR consumiu 8,9% menos dieta sólida do que o grupo BE CAR. Os animais AE CAR exibiram maior digestibilidade da proteína bruta e de extrato etéreo e tenderam a ter maior digestibilidade total da matéria seca e orgânica. Os animais do BE CAR apresentaram maior consumo de energia bruta e nitrogênio, porém, maiores perdas fecais tenderam a reduzir a eficiência no uso de energia e nitrogênio nesse grupo. A concentração total de AGV (µmol/mL), a proporção de acetato e propionato (% AGV) no fluido ruminal foram maiores para o grupo de BE em comparação ao grupo de AE. A concentração de ureia no sangue tendeu a ser maior em BE que em AE. As bezerras de AE e BE apresentaram valores de CGR de 0,080 e -0,077kg / d ($P \leq 0,01$), respectivamente. Os animais de AE para CGR exibiram maior ganho médio diário (9,4%), peso corporal e circunferência torácica, embora o grupo de AE para CGR tivessem um quadril mais estreito. Os

animais de AE para CGR tenderam a ter maior digestibilidade de extrato etéreo, mas maiores perdas de metano (% da energia bruta). Os animais AE CGR tenderam a ter maior proporção ruminal de acetato e menor propionato (% AGV). A concentração de insulina no sangue foi maior e a glicose sanguínea tendeu a ser maior para os animais BE que para os animais AE CGR. O grupo de BE CGR apresentou maiores temperaturas de costela e flanco esquerdo e superfície do ânus medida por termografia infravermelha em comparação ao grupo AE CGR. Maior eficiência em grupos de AE para CAR durante a fase de aleitamento parece estar relacionado a diferenças na digestibilidade, mas não a diferenças na fermentação ruminal. Diferenças no metabolismo de proteína parece afetar diferenças em CAR durante esta fase. Animais divergentes para CGR durante a fase avaliada parecem diferir em medidas corporais e no metabolismo de glicose e insulina, o que pode estar relacionado a diferenças na composição do ganho. A termografia infravermelha parece estar correlacionada ao CGR, mas não à produção de calor (**PC**) em bezerras durante a fase de aleitamento.

Palavras-chave: Consumo alimentar residual, ganho de peso residual, digestibilidade, produção de calor, parâmetros ruminais, metabólitos sanguíneos

ABSTRACT

The objectives of this study were: 1) to classify animals into groups of high and low feed efficiency using two feed efficiency indexes (residual feed intake (**RFI**) and residual feed intake and body weight gain (**RIG**)), and 2) to evaluate if pre-weaning heifer calves divergent for feed efficiency indexes exhibit differences in performance, body measurements, blood and ruminal parameters, digestibility, energy partitioning, nitrogen partitioning and thermography. 32 Gyr heifer calves were enrolled in a 63-d trial and classified into 2 feed efficiency (**FE**) groups based on RFI and RIG (mean ± 0.5 SD). The groups were classified as high efficiency (**HE**) RFI (HE RFI, n = 9; HE RIG, n = 10), and low efficiency (**LE**) RFI (LE RFI, n = 10; LE RIG, n = 11). HE and LE calves had RFI values of - 0.052 and 0.049 kg/d ($P < 0.05$), respectively). At birth, the amount of whole milk provided for each animal was calculated based on their metabolic weight at birth (42% x $BW^{0.75}$). The liquid diet was divided into two meals at 0700 and 1400. The total solid diet (**TSD**) was composed of 92% concentrate and 8% of *tifton* 85 hay chopped in 5 cm length as-fed. Intake was measured daily. Body weight and measurements were performed at birth, 3rd and 7th day and weekly onward. Blood concentrations of insulin, BHB, urea and glucose, and pH, N-NH_3 and volatile fatty acids (**VFA**) in ruminal fluid were evaluated at 14, 28, 42, 56 and 70 days of age, respectively. From the 50^{th} to the 55^{th} day of age the animals were submitted to a digestibility assay. At 55th (±6) days of age, respiratory exchanges were measured by one open-circuit respirometry chamber. Thermal images of the calves were taken with an infrared camera on d 56 (±3) at 0600 h, before the morning feeding. The HE RFI group consumed 8.9% less solid diet than the LE RFI group. HE RFI animals exhibited an increased digestibility of crude protein and ether extract and tended to have higher total dry and organic matter digestibility. LE RFI animals had higher gross energy and nitrogen intake, though higher fecal losses resulted in a tendency to reduce energy and nitrogen use efficiency. Total VFA concentration (µmol/mL), proportion of acetate and propionate (%VFA) in ruminal fluid were greater for LE than HE RFI heifers. Blood urea concentration tended to be higher in LE than HE RFI heifers. HE and LE calves had RIG values of 0.080 and -0.077kg/d ($P \leq 0.01$), respectively. HE RIG animals exhibited higher average daily gain (9.4%), body weight (**BW**), and heart girth, though HE RIG group exhibited narrower hip width. HE RIG animals tended to have higher ether extract digestibility but higher methane losses (% of gross energy). HE RIG animals tended to have greater ruminal proportion of acetate and lower propionate (%VFA). Blood insulin concentration was greater and blood glucose tended to be greater for LE than HE RIG animals. LE RIG had higher left rib, left flank and anus surface temperature

measured by infrared thermography in comparison to HE RIG group. HE RFI in pre-weaning heifers seems to be related to differences in digestibility and not related to differences in ruminal fermentation. Differences in protein metabolism seems to affect RFI during this phase. Divergent animals for RIG during the assessed phase appear to differ in body measurements, and glucose and insulin metabolism, which may be related to differences in the composition of the gain. Infrared thermography seems to be correlated to RIG but not to heat production (**HP**) in pre-weaning calves.

Keywords: residual feed intake, residual body weight gain, digestibility, heat production, ruminal parameters, blood metabolites

CAPÍTULO I – INTRODUÇÃO

1.1. Introdução geral

A alimentação em um sistema de produção de leite corresponde a 50% do custo total da produção (NASS, 2015) e por isso, a eficiência do uso dos alimentos pelos animais é imprescindível para aumentar a lucratividade (Pryce et al., 2014). Além disso, vacas mais eficientes têm menor emissão de metano (Hegarty et al., 2007), reduzindo o impacto ambiental.

A eficiência alimentar, definida como a fração da energia proveniente da alimentação que é transformada em produto, mais do que dobrou na indústria leiteira dos EUA nos últimos 100 anos (VandeHaar et al., 2016). O aumento da produção de leite por vaca é acompanhado do aumento no consumo, no entanto, maior proporção desses nutrientes é destinado a produção de leite ao invés de mantença e crescimento. Essa "diluição da mantença" é conseguida pelo maior consumo e produção de leite em animais do mesmo tamanho. Quando o aumento da capacidade de produção de leite advinda, exclusivamente do aumento do consumo de alimento e suplantada, o incremento na produção só ocorrerá acompanhado do aumento da estatura e do peso corporal, e dessa forma os ganhos por diluição da mantença serão menores ao longo do tempo (VandeHaar et al., 2016).

Aumentar a produção da vaca e o consumo de alimentos pode levar a redução total da digestibilidade do alimento (NRC, 2001), e aumento da excreção fecal de nutrientes, o que em média, aumenta a contaminação ambiental por unidade de consumo de matéria seca (Asher et al., 2014).

Uma alternativa para manutenção dos ganhos em eficiência alimentar ao longo do tempo é o uso do consumo residual alimentar (CAR) e ou ganho de peso residual (GPR), propostos por Koch et al. (1963). O CAR é uma medida fenotípica independente das características de produção, e dessa forma, animais mais eficientes apresentam menor consumo de alimentos sem comprometimento da produção. O GPR é uma medida de eficiência, que seleciona animais para maior ganho médio diário sem aumento no consumo de matéria seca.

No entanto, Berry e Crowley (2012) apontam como problema ao uso do CAR em animais em crescimento a possível seleção de animais de crescimento lento, e propõem para superar esse problema, a utilização do consumo e ganho residual (CGR) como índice alternativo. Portanto, a soma de mesmo peso de CAR e GPR pode ajudar a identificar animais

de melhor desempenho, associado a boa eficiência alimentar, sem perder a característica de ser hereditária. Segundo Berry e Crowley (2012) o CGR tem herdabilidade média (0,36 ± 0,06).

A definição de eficiência alimentar em vacas em lactação é mais complexa do que a para animais na fase de crescimento. Dentro das diferentes fases da lactação, as vacas passam por ciclos metabólicos caracterizados por rápido catabolismo das reservas corporais pós-parto, seguido por período de anabolismo das reservas corporais até o próximo parto (Roche et al., 2009). Qualquer índice de eficiência proposto deve levar em consideração a contribuição da mobilização de reservas corporais para o fornecimento de energia do animal (Berry e Crowley, 2013). A dificuldade da avaliação, e o maior custo para se avaliar grande número de animais em lactação fez com que as pesquisas de eficiência alimentar em bovinos leiteiros utilizassem a fase linear de crescimento como modelo para lactação (Waghorn et al., 2012).

Evidências indicam que bezerros da raça Holandês selecionados por divergência no CAR durante o crescimento exibem diferenças significativas (mesmo que reduzidas) na primeira lactação (Macdonald et al., 2014). Essa variação em CAR tem herdabilidade média (h2=0,45, Crowley et al., 2010), o que torna possível a seleção de animais de baixo CAR para compor o rebanho de reposição.

Caso as diferenças em CAR durante a fase de crescimento se mantenham nas fases subsequentes, a utilização de animais em crescimento como modelo para seleção de animais com baixo CAR permitirá selecionar animais mais eficientes precocemente reduzindo os gastos com a recria de animais menos eficientes que seriam descartados apenas no início ou durante a lactação.

Além disso, uma vez que as pesquisas evidenciam que o processo digestivo e as bases bioquímicas do metabolismo seriam em parte responsáveis pela divergência dos animais para eficiência (Waghorn e Dewhurst, 2007; Herd e Arthur, 2009), essas diferenças nas necessidades energéticas seriam igualmente aplicáveis às utilizadas para mantença tanto em animais jovens quanto em adultos. No entanto, isso pode não ser verdade para a eficiência de síntese de leite ou músculo, e por isso, mais estudos devem ser realizados comparando as medidas de eficiência nas diferentes fases da vida do animal.

Vários estudos foram realizados com o objetivo de avaliar e caracterizar o CAR de animais nas fases de recria e lactação (Waghorn et al., 2012; Macdonald et al., 2014), mas estudos que avaliaram essas características durante a fase de aleitamento são escassos.

Os animais da raça Gir tem grande contribuição para a pecuária leiteira brasileira uma vez que, 80% da produção de leite é proveniente de animais mestiços Holandês x zebu (Oliveira Júnior et al., 2017) nas suas diversas composições genéticas, sendo a raça Gir a mais utilizada

nos cruzamentos. O estudo eficiência alimentar na raça Gir é importante para que o melhoramento genético seja realizado nas duas raças parentais aumentando o potencial econômico dos animais cruzados.

O mecanismo biológico que controla o CAR não é completamente elucidado, mas inclui diferenças no processo fermentativo e digestivo, demanda fisiológica por nutrientes e no metabolismo pós-absortivo dos nutrientes ingeridos (Herd e Arthur, 2009). Ferramentas são necessárias para auxiliar os produtores a identificar animais mais eficientes na conversão de alimento em tecidos, sem, contudo, comprometer a produção de leite, o balanço energético no período de transição e a fertilidade nas vacas adultas. O estudo dos mecanismos fisiológicos relacionados a eficiência alimentar durante as fases iniciais de crescimento poderia auxiliar na previsão do desempenho desses animais na lactação.

CAPÍTULO II – REVISÃO DE LITERATURA

2.1. Importância da Raça Gir para a pecuária leiteira no Brasil

A produção de leite no Brasil a partir de animais *Bos Taurus* é um desafio principalmente devido aos altos índices de temperatura e umidade durante todo o ano. O estresse térmico causa prejuízos aos sistemas de produção, já que afeta o desempenho das vacas, reduz o consumo de matéria seca e a produção de leite, além de aumentar a incidência de doenças e afetar o desempenho reprodutivo (Tao e Dahl, 2013).

A produção de leite nos trópicos com o uso de raças especializadas exige grandes investimentos com infraestrutura, muito relacionada ao conforto térmico, além de alta dependência de alimentos conservados, o que nem sempre é viável economicamente. O desenvolvimento de tecnologias e modelos competitivos de produção que possam explorar as vantagens naturais do ambiente tropical pode aumentar a produção de leite no país e a lucratividade dos rebanhos (Ledic, 2008).

A utilização de animais cruzados, provenientes de acasalamentos entre animais *Bos Taurus Taurus* e *Bos Taurus Indicus*, é uma alternativa amplamente utilizada em países tropicais, tanto para produção de carne quanto de leite (Oliveira Júnior et al., 2017). Para vacas leiteiras, essa prática explora a complementariedade da alta produção dos taurinos e a tolerância ao calor e aos parasitas dos zebuínos.

A raça Girolando é resultante do acasalamento entre as raças Holandês e Gir, com composição genética variando de ¼ a 7/8 Holandês. No Brasil, terceiro maior produtor no

ranking mundial de produção de leite (FAO, 2017), 80% dessa produção é proveniente dos animais cruzados, principalmente da raça Girolando (Cole e Silva, 2016)

O Gir Brasileiro foi importado da Índia na década de 1930 e introduzido em rebanhos produtores de carne. Em 1960 alguns criadores de Gir começaram a selecionar esses animais para a produção de leite (Oliveira Júnior et al., 2017). Apesar dos animais importados serem relativamente poucos, esses animais se adaptaram muito bem ao ambiente brasileiro e se multiplicaram rapidamente (Santiago, 1985).

A raça Gir foi a primeira raça zebuína a ter programa genético para seleção de características leiteiras. A primeira avaliação genética de teste de progênie do Gir leiteiro foi publicada em 1993 pela EMBRAPA, (Empresa Brasileira de Pesquisa Agropecuária, Brasília, Brasil) e anualmente são realizadas novas avaliações (Verneque et al., 2014). A utilização de animais mestiços de qualidade no Brasil só ocorreu pelo melhoramento genético realizado tanto no Gir leiteiro quando na raça Holandês, já que permitiu associar características positivas para leite, persistência de lactação e rusticidade.

O estudo e desenvolvimento de índices de eficiência alimentar na raça Gir assim como no Holandês, é uma forma de melhorar fatores econômicos e ambientais, e aponta para uma oportunidade de melhoramento genético, aumentando o potencial econômico dos animais cruzados.

2.2. Índices de eficiência alimentar para animais em crescimento

O aumento da demanda por alimentos, devido ao crescimento da população humana, sem disponibilidade proporcional de terra agricultável, exigirá maior eficiência de produção de alimentos. A melhoria da eficiência alimentar nos animais via seleção genética é uma estratégia para minimizar esse problema, uma vez que é cumulativa e permanente (Berry e Crowley, 2013). Diante desse cenário, vários pesquisadores vêm estudando a melhor forma de calcular a eficiência alimentar e como utilizar esse índice na seleção genética.

Os índices de eficiência alimentar podem ser classificados de maneira simplificada em duas categorias: medidas de razão ou medidas de características residuais (Berry e Crowley, 2013). Um exemplo de uma medida de razão muito utilizada é a taxa de conversão alimentar, que é a razão entre o consumo de alimentos e o produto avaliado (ganho de peso ou produção de leite). Animais com a menor conversão alimentar são os mais eficientes.

A eficiência parcial de crescimento é outro índice usado, que consiste na proporção do ganho em relação ao consumo após serem descontadas as exigências de energia para mantença

(Berry e Crowley, 2013). As exigências de mantença podem ser calculadas utilizando tabelas como a do NRC (2001) a partir da média do peso corporal durante o período avaliado.

Apesar de serem amplamente utilizados, existem alguns problemas associados a esses índices que já foram amplamente discutidos (Veerkamp et al., 1995; Crews et al., 2006). Eles não levam em consideração as diferenças em eficiência para mantença, lactação, prenhez e ganho/perda de tecido corporal (Veerkamp et al., 1995), além de não considerarem as diferenças existentes em eficiência de mantença entre os animais (Berry e Crowley, 2013). Além disso, esses índices são fenotipicamente e geneticamente correlacionados com medidas de crescimento, produção e peso a idade adulta (Crews et al., 2006). Por isso, a seleção por essas variáveis resultará em altas taxas de crescimento, maior tamanho a idade adulta e consequentemente aumento das exigências de mantença (Crews et al., 2006), o que está em desacordo com o objetivo principal da seleção para melhor eficiência alimentar.

Dentro das medidas de características residuais, o índice mais usado é o consumo alimentar residual (CAR), também conhecido como eficiência alimentar líquida (Exton et al., 2000). O CAR pode ser definido como a diferença do consumo observado e o consumo predito. Como originalmente proposto por Koch et al. (1963), representa os resíduos de uma regressão de consumo de matéria seca em ganho médio diário (GMD) e peso metabólico (peso corporal elevado a 0,75) na metade do período de uma prova de eficiência (Crowley et al., 2010). Animais mais negativos para CAR são os mais eficientes durante o período de teste já que eles têm menor consumo do que o esperado para um animal com o ganho de peso observado.

Koch et al. (1963) também propuseram o ganho de peso residual como um índice alternativo para identificar variação na eficiência alimentar entre os animais. Utilizando um princípio similar ao do CAR, Koch et al. (1963) definiu o GPR como os resíduos de uma regressão do GMD em consumo de matéria seca e peso metabólico. Diferente do CAR, onde valores negativos indicam animais mais eficientes, o GPR considera como mais eficientes os animais que apresentam os valores mais positivos, uma vez que representam os animais que obtiveram ganho de peso maior que o predito para o consumo observado.

Berry e Crowley (2012) propuseram a criação de um índice alternativo a eficiência alimentar pela combinação do CAR e do GPR, o consumo e ganho residual (CGR). Eles padronizaram o CAR e o GPR para possuir a mesma variância e somaram as duas características após inverter o sinal do CAR. Assim, o valor positivo representa os animais mais eficientes.

Berry e Crowley (2012) citaram a falta de correlação entre CAR e ganho médio diário como uma possível razão da menor aceitação do CAR pela indústria, já que os indivíduos com crescimento lento poderiam estar no ranking dos mais eficientes. Esses autores ressaltam o

potencial desse novo índice em identificar animais que apresentam maior ganho médio diário com consumo proporcionalmente, menor que o esperado.

Para calcular o CAR, o consumo e o ganho médio diário devem ser medidos. O período de teste para se obter uma estimativa acurada de eficiência alimentar foi modificado ao longo do tempo. O período de teste de 112 dias anteriormente utilizado (Brown et al., 1991) foi reduzido para 70 dias, após adaptação, com a pesagens dos animais realizada pelo menos a cada duas semanas, sem que houvesse redução da eficiência do teste (Archer et al., 1997). Esses resultados sugerem que o fator limitante para determinar a eficiência alimentar é o intervalo de tempo necessário para medir a taxa de crescimento dos animais.

Dentro dessa perspectiva, Wang et al. (2006) realizaram um estudo com 456 novilhos para determinar a duração ideal do período de teste. Eles delinearam uma prova padrão de CAR, pesaram os animais semanalmente e avaliaram alterações relativas nas variâncias residuais fenotípicas e correlações (Pearson e Spearman) entre os dados de durações de teste encurtadas (7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 ou 84 d) e um teste de 91 dias. A conclusão foi que a prova de CAR poderia ser avaliada com 63 dias de duração se as pesagens corporais fossem realizadas semanalmente, sem comprometimento da acurácia.

Da mesma forma, Waghorn et al. (2012) realizando três pesagens por semana e conseguiram utilizar tempo de prova de 49 dias sem prejudicar a qualidade da avaliação, sugerindo que o aumento na frequência das pesagens pode reduzir ainda mais a duração dos ensaios de eficiência.

A necessidade de avaliar consumo diário de alimento e pesagens frequentes dos animais é fator limitante para o avanço nas pesquisas em eficiência alimentar. A maioria dos trabalhos realizados não consegue avaliar grande número de animais pelo custo desses experimentos. Dentro dessa perspectiva, a realização de trabalhos para tentar identificar marcadores de eficiência que substituam com acurácia as provas são importantes. Trabalhos realizados com o intuito de diminuir o tempo de prova também são importantes e o uso de novas tecnologias como alimentadores automáticos e plataformas de pesagem automáticas, que reduzem o tempo de deslocamento dos animais, tem potencial para facilitar e baratear as avaliações (Waghorn et al., 2012; Chizzotti et al., 2015).

2.3. Principais aspectos que contribuem para diferença em eficiência alimentar entre os animais

A independência do CAR da produção levou alguns autores a sugerir que o CAR é a variação inerente aos processos metabólicos (Herd e Arthur, 2009). Em termos gerais, é

provável que haja pelo menos cinco grandes processos pelos quais a variação da eficiência está associada: 1) ingestão de alimentos, 2) digestão de alimentos (e os custos energéticos associados), 3) metabolismo (anabolismo e catabolismo associado (incluindo variação em composição corporal), 4) atividade e 5) termoregulação (Herd et al., 2004).

A variação em consumo está associada a variação nas exigências de mantença dos animais. À medida que o consumo aumenta a quantidade de energia necessária para digerir o alimento também aumenta, em parte pelo aumento do tamanho dos órgãos digestivos e pelo aumento da energia para manutenção dos tecidos. Esse incremento calórico corresponde a aproximadamente 9% da energia metabolizável ingerida (Herd e Arthur, 2009).

O comportamento ingestivo, medido como taxa de ingestão e duração da refeição, também está relacionado com a eficiência alimentar (Adam et al., 1984). Animais de baixo CAR parecem passar menos tempo se alimentando por dia (Richardson e Herd, 2004) e estabelecem mais rapidamente um ciclo regular de ingestão de alimentos (Dobos e Herd, 2008).

A variação na digestibilidade total dos alimentos também parece existir entre animais que possuem divergência para características residuais de eficiência alimentar. Richardson et al. (1996) mostraram que touros jovens e novilhas ranqueados para alto e baixo CAR diferiram em aproximadamente 1% na sua habilidade de digerir os alimentos, o que explicou 14% das diferenças em consumo entre os dois grupos.

A deposição de quantidades iguais de tecido muscular e gordura têm influência na taxa e eficiência do ganho de peso devido ao menor teor de energia necessária para se acumular o mesmo peso de tecido muscular (Ferrell e Jenkins, 1998). Consequentemente, qualquer variação na composição do ganho pode influenciar a eficiência de utilização de nutrientes (Herd e Arthur, 2009). Novilhos selecionados para baixo CAR tem menor quantidade de gordura e maior acúmulo de proteína no corpo, mas essa diferença explica 5% da diferença de consumo entre os animais (Herd et al., 2004).

Basarab et al. (2011) recomendaram incluir avaliações ultrassonográficas da carcaça, para definição de ganho reservas proteicas ou lipídicas, no modelo de regressão múltipla para derivação do CAR como forma de retirar o efeito de composição corporal da comparação. Alguns trabalhos com animais de corte (Kelly et al., 2010a; b) já realizaram esse ajuste, assim como alguns trabalhos em vacas leiteiras incluem o escore de condição corporal (ECC) para considerar esse efeito (Hurley et al., 2016; Rathbun et al., 2017).

Os tecidos esplênicos que incluem o trato gastrointestinal (TGI), o fígado, o baço, o pâncreas e os depósitos de gordura mesentérica juntamente com seus tecidos conectivos e vasos sanguíneos associados, representam de 15 a 20% da massa corporal dos ruminantes (Seal e

Parker, 2000). O consumo de oxigênio para esses tecidos varia de 35 a 60% (Seal e Reynolds, 1993) e para o TGI sozinho esse valor corresponde aproximadamente a 20% (Cant et al., 1996). Richardson et al. (2001) concluíram que não existem diferenças em peso desses órgãos entre animais divergentes para CAR. As diferenças em ingestão de nutrientes entre animais com maior ou menor eficiência parecem estar mais relacionadas a processos metabólicos e não no tamanho dos órgãos por si só.

O consumo de oxigênio está diretamente relacionado a ingestão de alimentos (Huntington et al., 1988) e, portanto, é possível que os animais mais eficientes tenham menor consumo de oxigênio. Em novilhos, a taxa de respiração mitocondrial aumentou em animais mais eficientes e o fluxo de elétrons foi menor nos animais menos eficientes (Kolath et al., 2006), refletindo uma possível diferença em função mitocondrial.

As variações nos processos metabólicos podem afetar a produção de calor. A reciclagem de proteína nos animais é um processo de alto custo energético e parece ter relação com a eficiência alimentar dos animais. Richardson e Herd (2004) reportaram maiores concentrações de proteína plasmática e maiores concentrações sanguíneas de ureia e aspartato amino transferase em animais de alto CAR, o que relacionaram a uma possível variação de resposta ao estresse. Richardson et al. (2004) encontraram que animais de baixa eficiência (BE) para CAR tem maior cortisol plasmático e são mais susceptíveis ao estresse. Respostas fisiológicas ao estresse incluem aumento de taxa metabólica e consumo de energia, juntamente com o aumento dos processos catabólicos, como o aumento da lipólise e da degradação da proteína (Knott et al., 2008; Kelly et al., 2016), resultando em maior produção de calor e gasto energético por esses animais.

A variação em produção de calor e consequentemente de energia disponível para mantença e crescimento também ocorre como resultado de diferentes gastos energéticos associados com a atividade dos animais. Richardson et al. (1999) relataram correlação fenotípica de 0,32 para o CAR com a atividade, avaliada por pedômetro, o que indica que 10% da variação observada no CAR, sob as condições avaliadas, foram explicadas por essa medida de atividade.

Muitos trabalhos foram realizados na tentativa de entender os fatores que contribuem para as diferenças em eficiência alimentar. No entanto, os trabalhos avaliaram diferentes fases do desenvolvimento e apenas uma parte dos fatores que parecem explicar as diferenças em eficiência alimentar.

A avaliação isolada dos parâmetros metabólicos e corporais parece não ser a melhor maneira de entender as bases fisiológicas que explicam as divergências na eficiência alimentar. A avaliação das bases fisiológicas já associadas as alterações na eficiência alimentar em

experimentos com número de animais que possibilite a avaliação acurada dos dados ou a realização de metanálises que integrem os resultados já encontrados parecem ser a melhor abordagem para o entendimento dos fatores relacionados a variações na eficiência alimentar.

2.4. Parâmetros metabólicos e hormonais em bezerras em aleitamento e sua utilização em provas de eficiência alimentar

A criação de bezerras leiteiras, envolve o fornecimento de leite, na maioria das vezes de forma restrita, por um período limitado, até que o consumo de concentrado e forragem seja suficiente para permitir taxa de crescimento constante nesses animais (Hugi e Blum, 1997a). Existe grande diferença no metabolismo na fase de pré-ruminante e ruminante, e a mudança entre essas fases envolve, não apenas, modificações no trato gastrointestinal, mas também no sistema endócrino (Donkin e Hammon, 2005).

Muitos hormônios e fatores de crescimento estão envolvidos na regulação da absorção e distribuição dos nutrientes, e as diferenças metabólicas existentes entre os animais são determinadas pela genética, pelo ambiente e pela alimentação (Blum e Hammon, 1999). As diferenças metabólicas energéticas e proteicas, pré e pós-absorção podem afetar as taxas de crescimento corporal (Brickell et al., 2009). Isso é particularmente importante quando se avalia animais divergentes para CAR já que as diferenças endócrinas parecem ser uma das responsáveis por essa divergência. Kelly et al. (2010a) relataram que características comportamentais, composição de carcaça e mudanças em metabólitos circulantes em animais em crescimento são responsáveis por 35% da variação do CAR.

A glicose é um carboidrato monossacarídeo que é a maior fonte de energia para muitos organismos, incluindo o bezerro pré-ruminante. Concentrações plasmáticas de glicose podem ser influenciadas pelo consumo de matéria seca, síntese de glicose endógena via gliconeogênese e catabolismo do glicogênio estocado no fígado, músculo e tecido adiposo (Aronoff et al., 2004). A adequada regulação endócrina do metabolismo de glicose é crucial para a homeostase (Kaneko, 1997). Assim como em monogástricos, o primeiro substrato energético de bezerros em aleitamento é a lactose, que é derivada do leite ou sucedâneo (Davis e Drackley, 1998).

A glicemia dos bezerros em aleitamento nas primeiras semanas de vida fica em uma faixa que não é típica dos ruminantes variando de 80,7 mg/dl (Stern et al., 1970) a 108 mg/dl (Hugi e Blum, 1997b). O declínio da concentração de glicose no sangue ocorre nas primeiras semanas de vida, como resultado do desenvolvimento ruminal (McCandless e Dye, 1950).

Ao desaleitamento, os teores de glicose plasmática estão em torno de 69,3 mg/dl, um pouco mais alta do que em animais adultos, 64,6 mg/dl (Stern et al., 1970).

A insulina é um hormônio anabólico sintetizado pelas células beta nas ilhotas de Langerhans, localizadas no pâncreas (Kaneko, 1997). A função precípua da insulina é promover a absorção de glicose através das células, primariamente ativando transportadores de glicose (GLUT) (Kaneko, 1997). A produção e secreção de insulina depende da taxa de aparecimento de nutrientes no intestino delgado, composição do alimento (incluindo carboidratos e aminoácidos) e sinalização neuroendócrina para o pâncreas (Aronoff et al., 2004). Quando estimulada, a ação da insulina ocorre em duas fases: imediato aumento de insulina pré-formada e síntese contínua de insulina baseada na concentração da glicose plasmática (Aronoff et al., 2004). As concentrações de insulina reduzem com o aumento da idade e o aumento do consumo de alimento sólido pelos ruminantes (Benschop e Cant, 2009).

Com a mudança de pré-ruminante para ruminante funcional muitas mudanças ocorrem no metabolismo de energia dos animais. Ocorre mudança na fonte primária de energia de glicose, absorvida no intestino, para ácidos graxos voláteis (AGV) produzidos pela fermentação ruminal da dieta sólida consumida (Baldwin et al., 2004). Existem duas principais consequências da utilização de AGV como principal fonte de energia: o animal passa a não ter excesso de glicose na corrente sanguínea e o fígado remove quantidades insignificantes de glicose do sangue portal (Donkin e Hammon, 2005). O fígado do ruminante tem pouca ou nenhuma hexoquinase (Ballard, 1965), o que faz com que o fígado tenha sempre produção líquida de glicose (Ballard, 1965). Uma segunda adaptação diz respeito ao metabolismo lipídico. O ruminante usa apenas pequenas quantidades de glicose para a síntese lipídica, dessa maneira o acetato é o principal precursor da síntese de ácidos graxos (Hanson e Ballard, 1967; Donkin e Hammon, 2005).

A insulina é um importante regulador hormonal do metabolismo. Ela inibe a gliconeogênese hepática reduzindo a absorção hepática de alguns precursores de glicose e direciona o fluxo de nutrientes glicogênicos para os tecidos muscular e adiposo (Brockman e Laarveld, 1986). As altas concentrações de insulina promovem a síntese de proteínas e lipídios e o ganho corporal. Quando as concentrações de insulina são baixas, nutrientes são direcionados para o fígado e podem ser utilizados em maior taxa para a síntese de glicose (Brockman e Laarveld, 1986).

Richardson et al. (2004) relataram que a concentração de insulina plasmática apresentou tendência ($P < 0,10$) de ser mais elevada em animais de alto CAR. Kelly et al. (2010b) também relataram maior concentração de insulina em novilhas de terminação classificadas como alto CAR. Esses resultados indicam possível correlação entre a concentração plasmática de insulina e o CAR. Altas concentrações de insulina nos animais de alto CAR podem estar associadas com maior proporção de gordura, já que a insulina inibe a lipólise e estimula a lipogênese no tecido

adiposo (Hocquette et al., 1998). A insulina aumenta o transporte de aminoácidos para o músculo e a síntese de proteína, reduzindo a proteólise muscular (Hocquette et al., 1998), o que contraria a teoria da maior degradação proteica em animais de alto CAR. Richardson et al. (2004) hipotetizaram então, que animais classificados como alto CAR podem ter sensibilidade a insulina reduzida, o que reduz a ação anti-proteólise da insulina.

Kelly et al. (2010a) não encontraram diferenças em concentrações plasmáticas de insulina entre animais de alto e baixo CAR em novilhas em crescimento. Assim como Richardson et al. (2004) não foram encontradas diferenças na glicose basal.

Nascimento et al. (2015), trabalhando com animais nelore, encontraram maiores concentrações de insulina nos animais de menor CAR. A insulina é capaz de agir no hipotálamo através de receptores de glicose, provocando sinais de saciedade devido à homeostase de energia (Woods e D'Alessio, 2008). Xi et al. (2016) trabalhando com vacas Holandês, na metade da lactação, alimentadas com dieta completa, também não encontraram diferenças nas concentrações de glicose, beta hidroxibutirato (BHBA) e insulina. Zhang et, al. (2017) trabalhando com cordeiros em crescimento, alimentados com dieta peletizada, não encontraram diferenças no metabolismo de glicose.

A ureia é um produto da degradação proteica (Cameron, 1992). Na ausência de disfunções hepáticas e renais, as concentrações sanguíneas de ureia dependem da ingestão, síntese e degradação proteica (Kühne et al., 2000). A concentração de ureia aumenta em situações de alto consumo de proteína, uma vez que os aminoácidos em excesso serão utilizados como fonte de energia. Em ruminantes, o excesso de proteína degradada no rúmen (PDR) ou a limitação de energia disponível no rúmen resultará na não utilização da amônia pelos microrganismos ruminais e na absorção da amônia, pela parede ruminal, que será detoxificada a ureia no fígado. Durante período de restrição alimentar ou estresse, altas taxas de catabolismo proteico podem ocorrer, o que aumenta as concentrações de ureia circulante (Greenwood et al., 2002). Existe evidência que animais de alto CAR tem maior taxa de degradação proteica do que animais de baixo CAR (McDonagh et al., 2001). Animais menos eficientes parecem ser mais susceptíveis ao estresse e consequentemente tem maior mobilização proteica, o que pode contribuir para o aumento da ureia circulante (Kelly et al., 2016).

A redução da síntese proteica e altas concentrações sanguíneas de ureia podem ocorrer em animais alimentados com sucedâneo, mesmo com quantidades similares de nutrientes, mas sem os fatores de crescimento (Kühne et al., 2000), o que demonstra que o equilíbrio entre nutrientes e os fatores de crescimento é fundamental para a utilização da proteína em animais em desenvolvimento.

Kelly et al. (2010a) não encontraram nenhum efeito do CAR sobre a concentração plasmática de ureia. Richardson et al. (2004) e Nascimento et al. (2015) encontraram maiores concentrações sanguíneas de ureia nos animais menos eficientes. Isso pode estar relacionado à maior ingestão de proteínas, maior taxa de degradação proteica no corpo do animal, ou desvio no suprimento de aminoácidos por menor eficiência da produção de proteína microbiana no rúmen (Kahn et al., 2000; Herd e Arthur, 2009).

Richardson et al. (2004) citaram que as concentrações plasmáticas de ureia estão negativamente correlacionadas com deposição de tecido muscular e encontraram correlação positiva entre o CAR e os teores de ureia sanguínea em animais durante o desaleitamento. Os animais mais eficientes tenderam a ter menos gordura corporal e, por isso, diferenças em composição podem ter contribuído para essas associações.

O aumento no consumo de alimentos sólidos pelos bezerros causa algumas mudanças fisiológicas no animal. Em adição ao desenvolvimento físico do rúmen, ocorre aumento nas concentrações séricas de BHBA (Quigley, 1999). O BHBA é um metabólito importante conhecido como cetona, o que descreve sua composição química. Esse metabólito é usado pelo corpo como uma fonte de energia, e em ruminantes adultos é frequentemente produzido pela metabolização de gordura, que ocorre em fases de balanço energético negativo. Essa condição é chamada cetose e pode causar problemas clínicos nos animais (Quigley, 1999). Nos ruminantes adultos e, principalmente, nos animais que estão se tornando ruminantes a função ruminal e a produção de butirato pelo rúmen contribui para as concentrações plasmáticas de BHBA, o que dificulta o entendimento dos teores BHBA sanguíneo (Kelly, 1997).

No entanto, no animal jovem, o BHBA é usado como um indicador de desenvolvimento ruminal. Nas primeiras semanas após o desaleitamento, o consumo de alimento sólido está associado com o aumento na utilização do butirato que pode ser 60% maior do que a sua taxa de utilização em ruminantes adultos (Walker e Simmonds, 1962). O BHBA é oriundo do metabolismo do butirato, produzido pelas bactérias ruminais que fermentam os carboidratos a butirato no rúmen pelas células epiteliais do rúmen. O aumento do BHBA sanguíneo é importante para o animal já que o adapta para utilização dessa fonte de energia (Quigley, 1999), muito importante nos ruminantes.

A avaliação das concentrações plasmáticas de BHBA foi realizada em poucos trabalhos, em animais com rúmen funcional. Richardson et al. (2004) relataram correlação fenotípica positiva (r = 0,55) ao desaleitamento entre CAR e os teores plasmáticos de BHBA. Kelly et al. (2010a) também relataram correlação positiva entre BHBA e consumo de matéria seca (CMS) (r = 0,34) e entre BHBA e o CAR (r = 0,37). Existe relação entre maiores concentrações

plasmáticas de BHBA e maior CMS, no entanto não explica 100% da variação existente entre os animais divergentes para o CAR, o que indica que existem outras diferenças no metabolismo de energia entre animais com maior ou menor eficiência.

O BHBA circulante em ruminantes funcionais está relacionado a metabolização de ácidos graxos por mobilização de gordura corporal. Kelly et al. (2010b) encontraram associações negativas entre o BHBA e o acúmulo de gordura lombar subcutânea.

Muitos fatores podem estar relacionados ao estado metabólico do animal como a fase de crescimento ou de produção, a dieta a qual o animal está submetido e a composição corporal. Para que esses metabólitos possam ser usados como marcadores de eficiência, é importante que, independente das condições avaliadas, o comportamento seja igual para animais alta eficiência (AE) e BE, o que não aconteceu nos trabalhos revisados. Fatores de confundimento como a composição corporal, parecem contribuir com a diferença encontrada nos trabalhos e devem ser considerados nos futuros trabalhos para que marcadores do metabolismo relacionado a eficiência alimentar sejam identificados.

2.5. Desenvolvimento ruminal em bezerras em aleitamento

A fase de aleitamento é uma fase determinante para o desempenho dos animais já que nesse período ocorrem transformações que podem afetar a produtividade na vida adulta. Os quatro compartimentos do estômago de um ruminante (rúmen, retículo, omaso e abomaso) já estão presentes ao nascimento, mas o compartimento predominante é o abomaso (Warner et al., 1956). Os bezerros nascem com o rúmen fisicamente e metabolicamente não desenvolvido e inicialmente necessitam do leite para garantir as demandas de crescimento e mantença (Khan et al., 2016).

A transição de ruminante não funcional para ruminante funcional está relacionada a habilidade do rúmen de suportar a fermentação e essa capacidade é dependente de cinco elementos chave: estabelecimento da microbiota no rúmen, disponibilidade de substrato, presença de líquido, habilidade absortiva do tecido ruminal e fluxo de material do rúmen para o intestino (Gupta et al., 2016).

Os bezerros passam por três fases de desenvolvimento do rúmen que consistem em: Não ruminante (0-3 semanas de vida): Nessa fase o leite é o principal alimento. Ao final dela, o animal começa a ingerir em pequenas quantidades o alimento sólido e o rúmen começa a se desenvolver; Fase de transição: 3 a 8 semanas: A ingestão progressiva de alimento sólido, tanto a forragem como o concentrado estimulam o desenvolvimento da glândula salivar e do retículo-

rúmen; Ruminante (a partir da 8ª semana): O animal já começa a ter ciclos completos de motilidade ruminal e o rúmen está completamente desenvolvido. Seu crescimento continuará até 6 meses de idade e após esse período qualquer aumento no tamanho do órgão será proporcional ao tamanho total do animal (Gupta et al., 2016).

Na primeira semana de vida, o retículo-rúmen compreende 38% e o abomaso 49% do TGI dos bovinos. Na 8ª semana de idade o rúmen compreende 60% do TGI e o abomaso 27% (Church, 1979).

O desenvolvimento anatômico do rúmen inclui o crescimento do tamanho do órgão e o desenvolvimento das papilas ruminais (Reynolds et al., 2004). O leite ou sucedâneo, que é o principal alimento dos animais nas fases iniciais da vida, em condições normais, flui direto para o abomaso através do reflexo de formação da goteira esofágica (Church, 1979). Dessa forma o animal precisa ter acesso a alimento sólido de qualidade, que estimulará a fermentação ruminal, além da ingestão de água, uma vez que os microrganismos ruminais necessitam de ambiente aquoso para se desenvolver. Se a água não é fornecida separadamente do leite, o desenvolvimento ruminal pode ser reduzido (Lane et al., 2002).

Ao nascimento, já se inicia a colonização microbiana do rúmen. Os microrganismos de outros animais adultos e do ambiente colonizam o rúmen até que uma população microbiana diversa e complexa se estabeleça (Jami et al., 2013). Existe evidência de que a maioria dos microrganismos anaeróbios estritos que se tornam predominantes no rúmen do animal adulto, até mesmo os metanogênicos, já são presentes no rúmen um ou dois dias após o nascimento (Morvan et al., 1994). À medida que o animal começa a ingerir alimento sólido, a população microbiana aumenta e se assemelha a de um ruminante adulto (Greenwood et al., 1997) mudando em predominância de aeróbias para anaeróbias facultativas (Davis e Drackley, 1998).

O desenvolvimento funcional do rúmen envolve a capacidade de fermentação e de atividade enzimática (Faubladier et al., 2013). A absorção de AGV tem papel essencial no desenvolvimento das papilas ruminais (Van Soest, 1994). O poder estimulante de cada AGV é diferente, sendo o butirato o mais estimulador, seguido pelo propionato (Heinrichs, 2005). A baixa atividade de Acetil CoA sintetase parece limitar o metabolismo ruminal do acetato, limitando a capacidade do acetato de estimular o desenvolvimento epitelial (Harmon et al., 1991).

O desenvolvimento ruminal adequado exige que o material que entra no rúmen possa deixá-lo. A primeira contração ruminal ocorre por volta do 7º dia de vida. Esse é um fator chave no desenvolvimento do rúmen, que é obtido pela suplementação de alimento sólido em idade precoce (Gupta et al., 2016). Os concentrados estimulam primariamente o desenvolvimento do

epitélio (Jiao et al., 2015) e as forragens com alta proporção de fibra e tamanho adequado são os principais estímulos para o desenvolvimento muscular e o aumento do volume do rúmen (Žitnan et al., 1998).

Além de promover o desenvolvimento muscular, as forragens controlam o pH, estimulam o fluxo salivar para o rúmen e mantém a saúde do epitélio (Gupta et al., 2016). Se os bezerros forem alimentados apenas com concentrado, as papilas do rúmen podem ter crescimento exacerbado em resposta às altas concentrações de AGV, o que resultará em aglomeração de papilas e redução da área de superfície disponível para absorção (Gupta et al., 2016).

2.6. Avaliação de parâmetros ruminais em provas de eficiência alimentar

Herd e Arthur (2009) relataram que as diferenças no processo digestivo é um dos fatores que explicam as diferenças em CAR nos animais. Isso pode estar relacionado aos parâmetros de consumo, que afetam a taxa de passagem e a digestibilidade, mas fatores como o microbioma do animal podem estar envolvidos. As diferenças em digestibilidade podem representar 19% da variação fenótípica de CAR (Richardson e Herd, 2004), mas a dificuldade de detectar pequenas diferenças nos processos digestivos e disponibilidade de substrato dificultam sua detecção.

A fermentação ruminal é realizada por um microbioma diverso que consiste em bactérias, archaeas, protozoários e fungos. Independente da dieta, os AGV e a proteína microbiana (PM) são responsáveis por grande parte das exigências de energia e proteína do animal. Os AGV são absorvidos pelo epitélio e a PM é absorvida no intestino delgado. A amônia liberada pela degradação de proteína no rúmen é absorvida na parede ruminal para ser detoxificada no fígado a ureia, e posteriormente reciclados pelo corpo ou excretados na urina.

Hipotetiza-se que as diferenças no desenvolvimento inicial dos animais possam explicar diferenças no CAR. Isso pode estar relacionado as diferenças em colonização e desenvolvimento ruminal que afetarão os parâmetros fermentativos. Além disso, o estabelecimento do comportamento ingestivo dos animais pode afetar indiretamente a fermentação ruminal, pela associação negativa entre a taxa de alimentação e o tempo de retenção ruminal (Hegarty, 2004).

Guan et al. (2008) encontraram diferentes filotipos de bactérias no rúmen de novilhos classificados como alto e baixo CAR, sendo que filotipos específicos de bactérias parecem colonizar preferencialmente animais de baixo CAR. Outros estudos porém, relatam que o fenótipo microbiano do rúmen se altera nas diferentes dietas e que por isso esse parâmetro pode ser de menor importância no mecanismo biológico que controla o CAR (McCann et al., 2014).

Alguns estudos foram realizados para avaliar diferenças de consumo, digestão e parâmetros fermentativos em animais divergentes para CAR. No entanto, poucos trabalhos foram realizados em ruminantes em desenvolvimento.

Krueger et al. (2009a) não encontraram diferenças no pH ruminal de animais de alto e baixo CAR. Esses autores encontraram menores concentrações de propionato e maiores relações acetato:propionato para animais baixo CAR. McDonnell et al. (2016) também encontraram maior proporção acetato:propionato e menores concentrações de propionato em animais de baixo CAR alimentados com silagem de capim. No entanto isso não foi encontrado quando os animais estavam a pasto ou dieta total. Krueger et al. (2009b) não encontraram diferenças no pH ruminal ou concentrações de AGV entre os animais divergentes para CAR consumindo uma dieta de alto grão.

Lawrence et al. (2011, 2012) não encontraram diferenças no pH ruminal de vacas de corte gestantes de alto e baixo CAR alimentas com dieta a base de silagem de capim. Lawrence et al. (2011) e Fitzsimons et al. (2013) relataram que animais de baixo CAR tinham maiores concentrações de propionato e menores taxas acetato:propionato.

Guan et al. (2008) relataram que animais de baixo CAR tendem a ter maior concentração de AGV e maiores concentrações de butirato e valerato, comparado a animais de alto CAR. McDonnell et al. (2016) não encontraram diferenças em AGV total e em butirato mas o fluido ruminal dos animais de baixo CAR apresentou maior concentração de isovalerato.

Rius et al. (2012) alimentaram animais alto e baixo CAR com dietas similares e avaliaram consumo, digestão, retenção de N e ambiente ruminal. Embora não encontrassem diferenças no consumo, pH, perfil de AGV ou excreção urinária de N, vacas com baixo CAR tiveram maior digestibilidade do N e da matéria orgânica (MO) em adição às maiores concentrações de amônia no rúmen. Não foi encontrada relação entre o microbioma de rúmen e a eficiência alimentar. No entanto, a presença da bactéria *Lachnospiraceae* nos animais de baixo CAR foi associada com a maior eficiência.

Fitzsimons et al. (2014) estudando a relação entre CAR e desempenho, comportamento e fermentação ruminal relataram que o pH ruminal era menor para animais alto CAR. Animais baixo CAR tinham menor concentração de amônia no rúmen, diferente do encontrado por Rius et al. (2012) e nenhuma outra correlação entre fermentação ruminal e CAR foram detectadas.

Flutuações no pH ruminal podem ser determinadas pela taxa de passagem, frequência de alimentação e taxas de degradação da dieta (Allen, 1997). Diferentes cinéticas ruminais e aspectos fermentativos podem existir em animais de alto e baixo CAR. Carberry et al. (2012)

mostraram que, embora diferente para cada tipo de dieta, existe associação entre a diversidade microbiológica no rúmen e CAR.

Uma vez que o metabolismo tende a homeostase, as avaliações pontuais de hormônios e metabólitos podem ter poder limitado para responder a algumas perguntas sobre alterações no metabolismo. Com o avanço das tecnologias moleculares, estudos que avaliem a expressão gênica da produção de hormônios e enzimas chave, além de microbioma ruminal podem trazer respostas mais conclusivas sobre diferenças associadas a maior eficiência alimentar dos animais. O melhor entendimento do microbioma de rúmen, está auxiliando no melhor entendimento das funções ruminais. No entanto, a interpretação desses dados está em evolução, o que trará muitos benefícios nas publicações futuras.

2.7. Avaliações de desenvolvimento corporal e uso dessas avaliações com índices de eficiência alimentar

Tradicionalmente, as medidas de eficiência utilizadas são as medidas de razão onde se avalia o consumo e o ganho de forma direta. A taxa de conversão alimentar pode ser expressa como a razão entre o consumo e o ganho de peso ou a produção de leite. Sugeriu-se que a seleção para essa característica pode ser confundida com padrões de maturidade e tamanho corporal (Arthur et al., 2001), o que contribui para seleção de animais maiores e com maior exigência de mantença, o que não é interessante do ponto de vista econômico e ambiental.

No entanto, os mecanismos biológicos que afetam o CAR não estão totalmente elucidados e podem estar relacionados a mudanças de composição corporal nos animais. Medidas lineares e a ultrassonografia de carcaça mostraram-se preditores úteis de composição corporal e podem ser indicativos da partição de nutrientes (Kelly et al., 2011). Poucos trabalhos relacionaram o CAR com as medidas corporais lineares e os trabalhos existentes com animais de corte não identificaram diferenças entre os animais (Basarab et al., 2003; Nkrumah et al., 2007; Smith et al., 2010; Kelly et al., 2011).

Herd e Arthur (2009), em revisão, mostraram que as diferenças em animais de alto e baixo CAR podem refletir diferenças em composição do ganho, sendo que os animais de alto CAR, em algumas fases do desenvolvimento, podem ter maiores proporções de gordura na carcaça.

Em animais em crescimento esse efeito parece ser menos pronunciado, mas o uso de medidas lineares para avaliação entre esses animais pode ser útil para detectar possíveis diferenças de composição corporal entre os grupos.

2.8. Digestibilidade e partição de energia e nitrogênio em bezerras em aleitamento e uso dessas avaliações em provas de eficiência alimentar

A disponibilidade de nutrientes, e consequentemente de energia e nitrogênio disponível para um animal após o processo de digestão irá depender de vários fatores. Variações individuais das perdas ocorridas durante diferentes fases desse processo parecem ser responsáveis por diferenças em eficiência alimentar entre animais.

A energia bruta (EB) ingerida por um animal corresponde a 100% do potencial energético de um alimento, mensurado como a energia liberada na forma de calor quando uma substância e completamente oxidada a dióxido de carbono e água (Resende et al., 2011). Entretanto, perdas energéticas ocorrem durante o processo de digestão. A primeira grande perda de energia ocorre na forma de fezes, que subtraída da EB, é denominada energia digestível (ED).

Kenny et al. (2018) em revisão de literatura, citaram que o aumento do consumo de alimento pode reduzir a digestibilidade da dieta devido à redução no tempo de retenção da digesta no rúmen. Esse mecanismo poderia ser a causa de redução da digestibilidade em animais BE CAR. No entanto, Kenny et al. (2008) compilaram estudos comparando digestibilidade entre grupos AE e BE para CAR e 79% dos estudos citados não encontraram diferenças em digestibilidade entre grupos divergentes para CAR. Esses autores concluíram que não está claro na literatura se diferenças em digestibilidade são características inerentes ao CAR ou simplesmente uma função de taxa de passagem mais lenta devido a um menor consumo.

Estudos mais recentes que encontraram diferenças em digestibilidade entre grupos divergentes para CAR reportaram diferenças em eficiência da absorção intestinal dos nutrientes a nível molecular (Montanholi et al., 2013; Serão et al., 2013; Meyer et al., 2014) e microbiota (Vigors et al., 2016). Esses resultados demonstram que diferenças em digestibilidade em grupos divergentes para CAR podem não ser relacionadas apenas a diferenças em consumo.

Parte da ED é perdida na produção de urina e de gases oriundos da fermentação ruminal, como o metano. Esta energia perdida como urina e gases pode ser subtraída da energia digestível, obtendo-se a energia metabolizável (EM). A transformação de energia metabolizável para energia líquida e acompanhada de perdas na forma de calor proveniente do metabolismo dos alimentos e transformação dos nutrientes (Chwalibog, 2004). Subtraindo a produção de calor da EM temos a energia líquida (EL). A energia líquida representa a fração da energia que estará disponível para as funções de mantença, gestação, lactação e ganho de peso.

A produção de calor (PC) se deve às reações metabólicas associadas ao metabolismo de mantença, produção e outras funções como atividade física, regulação da temperatura corporal

e resposta imune (NRC, 2001). Por causa dos altos custos associados com o trato gastrointestinal e fígado, é provável que diferenças no tamanho e funcionalidade desses órgãos podem influenciar no requerimento energéticos do metabolismo basal. No entanto, dados publicados na literatura que examinaram variação no tamanho e processos metabólicos é inconsistente (Herd and Arthur, 2009; Kenny et al., 2018). Novos estudos que utilizam metodologias de base molecular sugeriram que diferenças em expressão gênica relacionados com processos metabólicos nos tecidos gastrointestinais podem desempenhar papel na divergência em eficiência alimentar (Paradis et al.,2015; Fitzsimons et al.,2017).

Em estudos de metabolismo energético, há grande interesse na mensuração da produção de calor com objetivo de obter informações sobre a eficiência metabólica do animal. O custo energético de mantença, perdido na forma de calor, pode representar até 75% do consumo total de energia (Ferrell and Jenkins, 1984). Nkrumah et al. (2006) relataram que a PC foi 21% menor para animais baixo CAR em relação ao alto CAR. Basarab et al. (2003) observaram produção de calor 9,3% menor em animais AE para CAR quando comparados com animais de BE. Com isso, esforço significativo vem sendo empregado na tentativa de desenvolver métodos que permitam estimar a PC em ruminantes e identificar fenótipos relacionados a eficiência alimentar que permitam a seleção de animais que perdem menor quantidade de energia na forma de calor e apresentem menor exigência para mantença (Bishop et al., 1991).

2.9. Termografia infravermelha e uso dessa avaliação em provas de eficiência alimentar

A tecnologia de termografia de infravermelho (TIV) é um método não invasivo que tem sido utilizado para indicar alterações biométricas térmicas no metabolismo animal resultantes do aumento da temperatura corporal e alterações no fluxo sanguíneo em resposta a condições ambientais ou fisiológicas (McManus et al., 2016). O infravermelho é uma frequência eletromagnética naturalmente emitida por qualquer corpo, com intensidade proporcional a sua temperatura. Assim, o termo visor facilita a localização de regiões quentes ou frias pela interpretação dos termogramas que fornecem imagens, em faixas de temperatura que podem variar de - 40 a 1500ºC (Colyn, 2013).

A TIV pode ter várias aplicações na pecuária. Pesquisadores já conseguiram relacionar a TIV com detecção precoce de doenças (Hurnik et al., 1984), qualidade de carne (Tong et al., 1995), e respostas relacionadas ao estresse (Stewart et al., 2008). Além disso, estudos

preliminares mostraram a possibilidade de usar a TIV para avaliar o CAR (Schaefer et al., 2005; Montanholi et al., 2007).

A aplicação da TIV em ensaios de eficiência alimentar è baseada na teoria de que animais mais eficientes tem menor requerimentos de energia basal (Nkrumah et al., 2006), e por isso uma menor quantidade de calor poderia ser dissipada pela superfície corporal (Kleiber, 1961). Corroborando com essa teoria, Schaefer et al. (2005) observaram que animais AE CAR tiveram menor temperatura dorsal máxima quando comparados com animais BE CAR. Montanholi et al. (2008) encontraram fortes correlações entre produção de calor medida em sistema de calorimetria indireta de circuito aberto e temperatura dos membros inferiores (r = 0,88, $P < 0,001$).

Além disso, Montanholi et al. (2009a) também encontraram que temperatura superficial medida por termografia foi responsável por 70% da variação em CAR entre animais AE e BE. Montanholi et al. (2009b) reforçaram que membro inferior e bochecha são as localizações que parecem ser mais promissoras para acesso indireto da eficiência alimentar em bovinos.

Martello et al. (2016) encontraram que, as temperaturas medidas por TIV do olho, bochecha, flanco, costelas, anca e pés dianteiro foram positivamente associados com frequência respiratória e temperatura retal. Maiores temperaturas foram associadas com AE CAR, o que foi associado com melhor eficiência dos mecanismos de termorregulação uma vez que a temperatura retal permaneceu mais baixa no grupo de AE CAR. A parte frontal da cabeça foi a área considerada mais promissora para se relacionar com CAR nesse estudo.

Leão et al. (2018) trabalhando com bezerras durante o aleitamento, encontraram menor produção de calor mensurada através do método de máscara facial em animais AE CAR quando comparado com animais de BE CAR. No entanto, não foram encontradas diferenças em temperatura superficial mensurada por TIV. Nenhuma diferença foi encontrada em produção de calor entre grupos divergente para ganho residual. No entanto, animais mais eficientes para ganho residual tiveram maior temperatura ocular quando comparado com animais de baixa eficiência para ganho residual.

Diferenças em temperatura superficial mensuradas por TIV parecem estar relacionadas com índices de eficiência alimentar. Entretanto, diferentes relações de TIV com temperatura corporal e produção de calor são encontradas na literatura, incluindo ausência de correlação entre temperatura superficial e produção de calor.

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3. CAPÍTULO III – ARTIGO I: PUBLICADO NA REVISTA PLOSONE

Relationship between feed efficiency indexes and performance, body measurements, digestibility, energy partitioning, and nitrogen partitioning in pre-weaning dairy heifers

3.1. ABSTRACT

The objectives of this study were: 1) to classify animals into groups of high and low feed efficiency using two feed efficiency indexes (Residual feed intake (**RFI**) and residual feed intake and body weight gain (**RIG**)), and 2) to evaluate if pre-weaning heifer calves divergent for feed efficiency indexes exhibit differences in performance, body measurements, digestibility, energy partitioning, and nitrogen partitioning. A total of 32 Gyr heifer calves were enrolled in a 63-d trial and classified into two feed efficiency (**FE**) groups based on RFI and RIG (mean ± 0.5 SD). The groups were classified as high efficiency (**HE**) RFI (HE RFI, n = 9; HE RIG, n = 10), and low efficiency (**LE**) RFI (LE RFI, n = 10; LE RIG, n = 11). The remaining animals were classified as intermediate (n = 13 (RFI) and n = 11 (RIG)). HE and LE calves had RFI values of - 0.052 and 0.049 kg/d ($P < 0.05$), respectively. The HE RFI group consumed 8.9% less solid diet than the LE RFI group. HE RFI animals exhibited an increased digestibility of crude protein and ether extract and tended to have greater total dry and organic matter digestibility. LE RFI animals had greater gross energy and nitrogen intake, though greater fecal losses resulted in a tendency to reduce energy and nitrogen use efficiency. HE and LE calves had RIG values of 0.080 and -0.077kg/d ($P \leq 0.01$), respectively. HE RIG animals exhibited greater average daily gain (9.4%), body weight (**BW**), and heart girth, though HE RIG group exhibited narrower hip width. HE RIG animals tended to have greater ether extract digestibility but greater methane losses (% of gross energy). HE RFI in pre-weaning heifers seems to be related to differences in digestibility. Divergent animals for RIG during the assessed phase

appear to differ in body measurements, which may be related to differences in the composition of the gain.

Keywords: Residual feed intake, body weight gain, digestibility, heat production, nitrogen efficiency.

3.2. INTRODUCTION

The efficiency of an animal in converting feed into products is influenced by genetic, physiological, and environmental factors that result in individual variation in energy expenditure [1].The utilization of feed efficiency (FE) indexes aims to identify and select animals with great economic value. The greatest challenge in using such indexes involves determining which traits to include in the index and how to weigh them in order to maximize economic gain [2].

Residual feed intake (**RFI**), calculated by the difference between actual and expected animal feed intake is a feed efficiency (**FE**) index that is widely used in beef cattle, with the difference between animals reflecting inherent metabolic differences [3]. A previous study showed that the selection of slow-growing animals can be a problem associated with using RFI as an FE index, and have proposed the use of residual intake and body weight gain (**RIG**) as an alternative index in growing animals [4]. However, we are unaware of any existing work that has evaluated the use of RIG as an efficiency index in pre-weaning heifers and the possible impacts of this on physiological and productive parameters.

Differences in digestion and energy use can be important factors affecting FE. Lesser FE may be related to greater nutrient losses and greater methane yield. Greater maintenance costs can be also associated with high heat production by animals [5], though the importance of these factors influencing FE in the pre-weaning phase has not been determined.

Thus, the objectives of this study were: 1) to classify animals into groups of high and low FE using two FE indexes (RFI and RIG), and 2) to evaluate whether pre-weaning heifer calves divergent for FE indexes exhibit differences in performance, body measurements, digestibility, energy partitioning, and nitrogen partitioning.

3.3.MATERIAL AND METHODS

3.3.1. Calves, housing, management, and treatments

This study was approved by the Ethics Committee of Embrapa Dairy Cattle (number: 7194210316). The experiment was conducted at the Experimental Farm of Embrapa Dairy Cattle, located in Coronel Pacheco, Minas Gerais, Brazil.

A total of 32 Gyr heifer calves produced by in vitro fertilization and born during the autumn (April to June) were used. After birth, the animals were immediately separated from their dams, weighed, and had the umbilical cord immersed in iodine solution (10%).

Colostrum was administered (10% of BW; >50g of IgG L) up to 6 hours after birth. Blood samples were collected via jugular venipuncture up to 48 hours post-birth to measure total plasma protein (g/dL) using an electronic refractometer (Serum protein REF-301, Biocotek, Beilun, Ningbo, China).

Samples were centrifuged at 1,800 x g for 10 minutes at room temperature (22-25 °C). Total serum protein > 5.5 g / dL was used as a threshold for good transfer of immunity [6]. The heifers were housed in a shed without lateral walls, in individual sand beds (1.25 x 1.75 m) contained by chains 1.2 m in length.

The amount of milk offered for each heifer was based on their metabolic weight at birth. The amount of milk routinely supplied in Brazilian farms is 6 L of whole milk for animals with a mean birth weight of 35 kg [7], was used as a reference. This equates to 42% of the metabolic weight at birth in liters of milk. The objective was to standardize the amount of nutrients

supplied to the heifers from the liquid diet. The mean weight of the heifers at birth was 25.2 ± 3.2 kg (mean ± SD). Consequently, the daily milk supply was 4.7 ± 0.46 L.

The volume of milk provided to the heifers was constant throughout the experiment. During the pre-weaning period, heifers received a liquid diet divided into two equal meals offered at 0700 and 1400 in nipple buckets (Milk Bar®, New Zealand). Heifers received transition milk until 3 days of age, and whole milk from the 4th to the 77th day of age. On the 78th day of age, milk supply was reduced by half and animals were weaned on the 81st day of age.

Water and solid diet were offered *ad libitum* in buckets (10% of refusals for solid diet). This solid diet was composed of 92% starter (Soymax Rumen pre-initial, 18% flocculated, Total Alimentos, Três Corações, Minas Gerais, Brazil) and 8% Tifton 85 (*Cynodon* spp) hay chopped in 5 cm length, as fed (Table 1).

Table 1. Nutritional composition (DM basis, % unless otherwise noted) of hay, starter, and total solid diet (TSD, 92% concentrate and 8% hay) offered to calves during the pre-weaning, from 14 to 77 days of age

Nutritional composition	Hay	Starter	TSD[1]
DM[2]	79.4	84.3	84.0
CP[3]	10.0	19.3	18.5
OM[4]	74.0	78.8	78.4
EE[5]	3.27	3.33	3.33
NDF[6]	75.8	28.8	32.5
GE[7] (Kcal/kg)	4141	4243	4235

[1]TSD = total solid diet

[2]DM = dry matter

[3]CP = crude protein

[4]OM = organic matter

[5]EE = ether extract

[6]NDF = neutral detergent fiber

[7]GE = gross energy.

3.3.2. Handling and health parameters

On the 10th day of life, an oral anticocciodiostatic was administered to the animals (Isocox, Ouro Fino Saúde Animal, Cravinhos, São Paulo, Brazil) at a dose of 3 mL per kg of BW. Fecal scores were evaluated as follows: 1–normal (firm, but not hard); 2–soft (does not hold form, piles but spreads slightly); 3–runny (spreads readily to approximately 6 mm depth); and 4–watery (liquid consistency, splatters) [8]. A heifer was considered to have diarrhea if the fecal score was 3 or 4, and this condition was treated according to the farm protocol. All the episodes of diarrhea occurred within the first two weeks of life and did not influence the calculation of FE indexes.

All heifers were dehorned at 35±3 d of age using hot iron. They received local anesthesia (5.0 mL/horn, Lidovet, Bravet, Engenho Novo, Brazil) prior to the procedure and two days of non-steroid anti-inflammatory (0.025 mL/kg, Maxicam 2%, Ouro fino, Cravinhos, Brazil).

Nutrient composition analysis

Milk samples were collected twice daily (morning and afternoon) and analyzed for total solids, crude protein (**CP**), lactose, and fat. Milk component analysis was performed using an infrared analyzer (Bentley model 2000, Bentley Instruments Inc., Chaska, MN, USA). Mean ± SD values for the milk analysis were: 12.9% ± 1.1 for total solids, 4.4% ± 1.0 for fat, 3.1% ± 0.1 for CP, and 4.5% ± 0.1 for lactose.

Samples of the solid diet (**TSD**; hay and starter) were collected three times a week and homogenized weekly in a pool. Individual refusals were collected daily and were also homogenized in weekly a pool. Samples were stored at -20° C until processing. Feed samples were oven dried at 55 °C for 72 hours, ground through a 1 mm sieve in a Wiley type mill (model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) and analyzed for dry matter (**DM**), CP, ether extract (**EE**), ash [9] and neutral detergent fiber (**NDF**) [10]. Gross energy was determined using an adiabatic calorimeter (IKA - C5000, IKA® Works, Staufen, Germany).

3.3.3. Intake, performance, and body measurements

Milk, TSD, and water intake were measured individually. Daily intakes of milk were measured by the difference between offers and the refusals of the two meals (0700 and 1400). Water and TSD intake were calculated by the difference between offers and refusals measured 0900 daily. Feed and water were weighed using a bench scale (9094 plus, Toledo®, São Bernardo do Campo, São Paulo, Brazil) and a portable scale (WH-A04, WeiHeng, China), respectively. Scales had a precision of 0.1 g and 10 g, respectively.

Weight and body measurements were performed before the morning meal on days 3 and 7 after birth, and weekly from day 8 onward. Body weight was measured using a mobile mechanical scale (ICS-300 Móvel Mecânica, Coimma®, Dracena, São Paulo, Brazil) with precision of 0.1 kg. Withers height and hip height were measured using a measuring stick (Walmur, Porto Alegre, RS, Brazil). Hip width and heart girth were measured using a measuring tape (Bovitec, São Paulo, SP, Brazil).

3.3.4. Feed efficiency indexes

Solid feed was offered since the first day of life, but feed efficiency evaluations started with 14 d of age since there was no expressive solid intake before this age. Intake and performance were evaluated from the 14^{th} to the 77^{th} day of age, and the indexes were calculated based on 63 days of observation [11].The growth rate of the animals was modeled by linear regression of BW against time over the trial duration, and the regression coefficients were calculated for the average daily gain (**ADG**) of each animal. Mean daily feed intake was calculated for each animal over the trial period and corrected for DM. The average metabolic weight ($BW^{0.75}$) was calculated using the BW at the 46^{th} day of age, which was the middle of the test period.

Dry matter intake, $BW^{0.75}$, and ADG were used to estimate RFI and residual body weight gain using linear regressions [3], where RFI and residual body weight gain (**RG**) were

calculated as the difference between actual and predicted DMI and ADG, respectively, as follows:

$$Y_j = \beta_0 + \beta_1(BW^{0.75}{}_j) + \beta_2(ADG_j \text{ or } DMI_j) + e_j,$$

where Yj is the standardized DMI (RFI) or ADG (RG) of calf j, β0 is the intercept, β1 is the regression coefficient for $BW^{0.75}$, β2 is the regression coefficient for ADG (RFI) or DMI (RG), and ej is the error term for calf j.

In the present study, RG was not used as an FE index. The RG calculation was performed to estimate RIG.

To calculate RIG, the residues for RFI and RG were added as [4]:

$$RIG = [RFI \times (-1)] + RG$$

Based on these indexes, the animals were classified into four groups: high efficiency (**HE**) and low efficiency (**LE**) for RFI and RIG. HE indicated RFI < 0.5 SD below the mean (n = 9) and RIG > 0.5 SD above the mean (n = 10), while LE indicated RFI > 0.5 SD above the mean (n=10) and RIG < 0.5 SD below the mean (n = 11). The remaining animals were classified as intermediate and were not included in subsequent analyses. The feed efficiency indexes, DMI, BW, and ADG of the high and low efficiency groups are presented in Table 2.

Table 2. Intake, performance and body measurements in pre-weaning calves (14 to 77 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI and RIG

Item	RFI[1]			*P*-value	RIG[2]			*P*-value
	HE[3]	LE[4]	SEM	G[5]	HE	LE	SEM	G
RFI	-0.052	0.049	0.01	<0.01				
RIG					0.080	-0.077	0.018	<0.01
Intake (Kg/d)								
Water	0.33	0.38	0.07	0.67	0.35	0.38	0.03	0.41
Solid diet	0.16	0.24	0.02	0.001	0.17	0.20	0.01	0.07
Milk	0.61	0.60	0.003	0.07	0.60	0.61	0.001	0.61
Total	0.76	0.84	0.02	0.002	0.78	0.80	0.01	0.08
Body measurements								
ADG[6] (kg/d)	0.60	0.59	0.02	0.67	0.60	0.55	0.02	0.04
Whither height (cm)	84.5	85.2	0.40	0.21	84.5	84.7	0.23	0.46
Hip height (cm)	88.5	88.9	0.50	0.57	88.2	88.5	0.22	0.37
Body weight (kg)	48.2	46.6	0.77	0.16	47.8	46.3	0.25	<0.001
Hip width (cm)	22.2	22.6	0.13	0.06	22.0	22.5	0.10	<0.001
Heart girth (cm)	79.3	78.3	0.48	0.17	79.1	77.8	0.17	<0.001

[1]RFI = Residual feed intake

[2]RIG = Residual intake and gain

[3]HE = High efficiency

[4]LE = Low efficiency

[5]G = Main effect of group. Group × period interaction, $P \geq 0.06$, except solid diet intake and total intake for RFI groups ($P < 0.01$) and milk intake and heart girth for RIG groups ($P < 0.05$) (Figs 1 and 2, respectively)

[6]ADG = Average daily gain

3.3.5. Whole tract digestibility

From the 50th to the 55th day of age, collections of fecal and urine were performed. They were housed in metabolic cages (dimensions of 1.50 m × 0.80 m; Intergado Ltda., Contagem, Brazil) for two consecutive days for urine and feces collection. The urine tray was designed

with inclination to drain urine into 5 L containers stored in expanded polystyrene thermal boxes with ice. The volumes, weights, and urine densities of each animal were measured every 24 hours, and one 50 mL pure urine sample was taken after being filtered through cheesecloth and stored at -20°C.

After 2 days in metabolic cages, the animals were transferred into tie-stalls with a rubber mat for another 3 days of fecal collection. During these 5 days, the total fecal excretions of the animal were collected and weighed at least three times per day. Equivalent quantities of the daily sub-samples were combined into one sample per animal.

Samples of starter, hay, and orts were also collected daily, during the 5 days, from each animal. At the end of the collection period, equivalent quantities of the daily sub-samples were combined into one sample per animal and frozen at -20°C for further analysis. After thawing, feed, orts, and feces samples were oven dried at 55°C for 72 hours, ground through a 1 mm sieve in a Wiley type mill (model 3, Arthur H. Thomas Co., Philadelphia, PA), and analyzed for DM, CP, EE, ash, NDF, and gross energy (as explained in the nutrient composition analysis section). Urine nitrogen content was obtained by the Kjeldahl method [9], and gross energy was determined using an adiabatic calorimeter (IKA - C5000, IKA® Works, Staufen, Germany).

3.3.6. Respiratory exchanges and methane emission

On the 55^{th} (±6) day of age, respiratory exchanges and methane emissions were measured using an open-circuit respirometry chamber (Intergado® Ltda., Contagem, MG, Brazil) with a volume of 6.39 m^3 (2.48× 1.48 × 1.74 m) made from aluminum and transparent polyethylene terephthalate glycol (PETG) walls.

Animals were housed in a metabolic cage (dimensions of 1.50 mx 0.80 m; Intergado Ltda., Contagem, Brazil) allocated inside the respirometry chamber. The air inside the chamber was maintained at 60% RH and 20°C. A mass flow meter (Flow Kit model FK-500, Sable International Systems, Las Vegas, NV) continuously pulled air from the chamber (100 L/min).

Air from the chamber and ambient air were analyzed for a gas analysis and data acquisition system (13) to monitor O_2, CO_2, and CH_4 concentrations by the analyzers FC-10 oxygen, CA-10 carbon dioxide and MA-10 CH_4 (Sable International Systems, Las Vegas, NV). Total gas exchange for O_2, CO_2, and CH_4 were calculated [12].

The same procedure applied for the digestibility trial was used to calculate the DMI inside the chamber. The animals were weighed before entering the chamber, and the urine volume produced inside the chamber was recorded.

Calculations

The dry matter intake of each nutrient was calculated as the sum of intake (as fed) of each of the supplied components (milk, starter, and hay) and their respective content of DM and nutrients, discounting the quantities of DM and nutrients obtained from the orts of the milk and TSD.

Apparent digestibility values (%) were determined using the amount consumed and the amount of each nutritional component recovered in feces. Nitrogen balance was calculated by the difference between nitrogen intake in the diet and nitrogen excreted in feces and urine.

Gross energy intake (**GEI**) was calculated by the difference between the gross energy (**GE**) content of each of the supplied components (milk, starter, and hay) and those obtained in orts. GE content of milk was calculated as [13]: GE (Mcal/kg milk) = (0.0911 x % fat) + (0.0586 x % protein) + (0.0395 x % lactose).

Digestible energy intake (**DEI**) was calculated by the difference between GEI and fecal energy excretion. Subsequently, metabolizable energy intake (**MEI**) was calculated as the difference between DEI and the sum of urine energy and CH_4 energy, which was assumed to be 39.5 kJl/L [14]. Heat production (**HP**; kJ/d) was determined based on measurements of O_2 consumption (L/d), CO_2, and CH_4 production (L/d), and urine N output (g/d) applying the equation of Brouwer [14]. Energy balance (**EB**) was calculated as the difference between MEI

and heat production. Percentages of energy loss through feces, urine, methane, and heat (% GEI), as well as the relationships between ME/GE, DE/GE, HP/ME, and EB/ME were calculated and used as indicators of energy efficiency.

3.3.7. Statistical analyses

The statistical analyses were performed using SAS software [15]. To evaluate the effects of efficiency in the groups, the MIXED procedure was used for intake, performance, and body measurements, according to the model:

$$Y_{ijk} = \beta_0 + \beta_1 A_{ij} + \beta_2 B_{ij} + G_i + M_k + GM_{ik} + \delta_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable; β_0 is the intercept; $\beta_1 A_{ij}$ is the regression coefficient for the covariate initial BW; $\beta_2 B_{ij}$ is the regression coefficient for the covariate total serum protein; G_i is the fixed effect of efficiency group (RFI or RIG); M_k is the fixed effect of repeated measure (day or week); GM_{ik} is the fixed effect of interaction between group and repeated measure; δ_{ij} is the random error between animals within treatment; and ε_{ijk} is the random error between measurements among animals. The best covariance structure for repeated measures was chosen by the lowest corrected Akaike information criteria. The covariance structures evaluated were: variance components, composed symmetry, heterogeneous composed symmetry, autoregressive, heterogeneous autoregressive, and unstructured. For most of the dependent variables the heterogeneous composed symmetry structure was selected. For significant interaction between group and repeated measure, the differences among groups within measures were evaluated using the SLICE statement.

To evaluate data on digestibility, gas exchange, HP, energy intake, energy losses, energy use efficiency, and nitrogen balance, the following model was used:

$$Y_{ijk} = \beta_0 + \beta_1 A_{ij} + \beta_2 B_{ij} + G_i + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable; β_0 is the intercept; $\beta_1 A_{ij}$ is the regression coefficient for the covariate initial BW; $\beta_2 B_{ij}$ is the regression coefficient for the covariate total serum protein; G_i is the fixed effect of efficiency group (RFI or RIG); and ε_{ijk} is the random error. Pearson correlation coefficients between the response variables and RFI and RIG were obtained with PROC CORR. Significance of the effects was declared at $P \leq 0.05$ and tendency was accepted when $0.05 < P \leq 0.10$. Covariates were removed from the model when not significant.

3.4. RESULTS

3.4.1. Intake and performance – RFI

Divergence was observed among the animals for RFI during the pre-weaning phase (Table 2). The average RFI for the HE group was -0.052 kg/d and was 0.049 kg/d for the LE group. HE RFI animals consumed 8.9% less than LE RFI animals.

Treatment × week interaction was observed for solid diet intake and total intake (Table 2; Fig 1). Solid diet intake was greater ($P \leq 0.05$) for the LE RFI group from weeks 4 to 11, except for week 10 ($P = 0.06$). Total diet intake was greater ($P \leq 0.05$) for the LE RFI group from weeks 5 to 11, except for weeks 9 and 10 ($P = 0.07$ and $P = 0.09$, respectively).

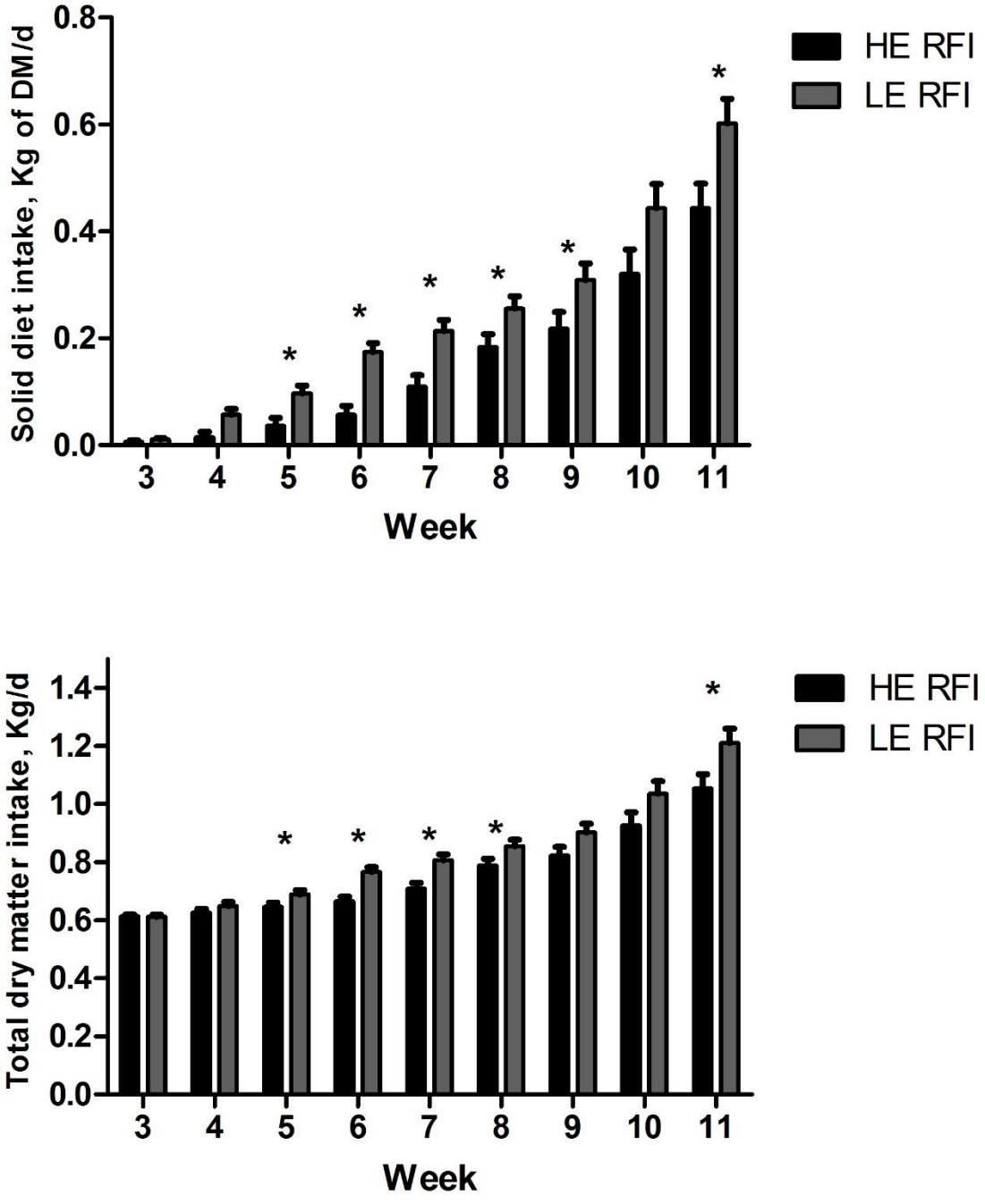

Fig 1. Weekly solid diet intake and total dry matter intake in pre-weaning calves (14 to 77 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI

No differences in water intake were observed, though there was a tendency for greater milk intake among HE RFI animals ($P = 0.07$).

No differences in BW, ADG, withers height, hip height, and heart girth were observed between the groups ($P \geq 0.16$). However, there was a tendency for greater ($P = 0.06$) hip width among LE RIF animals.

3.4.2. Digestibility – RFI

HE RFI animals exhibited an increased digestibility of CP ($P = 0.02$) and EE ($P = 0.02$) and tended to have improved total DM ($P = 0.06$) and organic matter (**OM**) ($P = 0.07$) digestibility. No differences in NDF digestibility were observed (Table 3).

Table 3. Digestibility (%) of DM, OM and nutrients in pre-weaning calves (50 to 55 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI and RIG

Item	RFI[1]			*P*-value	RIG[2]			*P*-value
	HE[3]	LE[4]	SEM	G[5]	HE	LE	SEM	G
DM[6]	89.2	85.8	1.24	0.06	88.1	87.4	1.28	0.70
OM[7]	91.1	88.0	1.15	0.07	90.2	89.5	1.20	0.68
CP[8]	91.8	87.8	1.16	0.02	90.8	88.5	1.18	0.18
NDF[9]	52.7	40.5	7.56	0.27	48.0	45.7	6.37	0.80
EE[10]	96.4	93.1	0.95	0.02	96.3	93.9	0.90	0.07

[1]RFI = Residual feed intake

[2]RIG = Residual intake and gain

[3]HE = High efficiency

[4]LE = Low efficiency

[5]G = Main effect of group

[6]DM = dry matter

[7]OM = organic matter

[8]CP = crude protein

[9]NDF = neutral detergent fiber

[10]EE = ether extract

3.4.3. Energy intake, losses and energy use efficiency - RFI

Positive correlations were observed between RFI and GEI (r = 0.50; *P* = 0.03), indicating that high RFI values (LE RFI) are correlated with higher gross energy intake. HE RFI animals also exhibited lower GEI but similar DE, ME, and NE intake (Table 4).

Table 4. Energy intake, losses and energy use efficiency in pre-weaning calves (50 to 55 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI and RIG

Item	RFI[1]			*P*-value	RIG[2]			*P*-value
	HE[3]	LE[4]	SEM	G[5]	HE	LE	SEM	G
Energy intake (kJ/d/BW$^{0.75}$)								
Gross energy	1000	1112	3.30	0.03	992	1063	3.56	0.17
Digestible energy	925	996	3.10	0.11	912	975	2.85	0.12
Metabolizable energy	908	979	3.10	0.12	891	958	2.80	0.11
Energy balance	305	356	3.22	0.28	290	336	2.85	0.25
Energetic outputs (% GE)								
Feces	7.66	10.6	1.06	0.06	8.22	8.35	0.85	0.91
Methane	0.17	0.22	0.08	0.66	0.22	0.11	0.04	0.05
Urine	1.36	1.39	0.13	0.86	1.39	1.37	0.14	0.92
Heat	60.9	56.1	2.26	<0.001	61.3	59.1	2.42	0.51
Energy balance	30.0	31.6	2.33	0.61	28.8	31.1	1.89	0.40
Energy use efficiency (%)								
DE:GE[6]	92.3	89.4	1.06	0.06	91.9	91.9	0.85	0.91
ME:GE[7]	90.8	87.8	1.10	0.06	90.2	90.2	0.84	1.00
HP:ME[8]	67.0	64.0	2.50	0.39	68.0	65.4	2.26	0.42
EB:ME[9]	33.0	36.0	2.50	0.39	32.0	34.6	2.26	0.42

[1]RFI = Residual feed intake

[2]RIG = Residual intake and gain

[3]HE = High efficiency

[4]LE = Low efficiency

[5]G = Main effect of group

[6]DE:GE: Ratio between digestible energy and gross energy

[7]ME:GE: Metabolizability

[8]HP:ME Ratio between heat production and gross energy

[9]EB:ME = Ratio between energy balance and gross energy

Energy losses in feces were also positive correlated with RFI (r = 0.41; *P* = 0.08). LE RFI animals had a greater DMI but tended to have greater fecal losses (*P* = 0.06). No differences in energetic losses in urine and methane emissions were observed between HE and LE RFI animals. HE RFI animals exhibited greater (*P* < 0.001) HP as a percentage of GEI.

HE RFI animals tended to have greater DE:GE (%) and ME:GE (*P* = 0.06) though with the same EB (% GE) when compared to LE RFI animals.

3.4.4. Nitrogen balance - RFI

Greater (*P* = 0.05) nitrogen intake and greater (*P* = 0.02) nitrogen loss in feces were observed in LE RFI animals. However, no differences were observed in urine nitrogen losses and retained nitrogen between the groups (*P* ≥0.50) (Table 5).

Table 5. Nitrogen balance (g/d/$BW^{0.75}$) in pre-weaning calves (50 to 55 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI and RIG

Item	RFI[1]			*P*-value	RIG[2]			*P*-value
	HE[3]	LE[4]	SEM	G[5]	HE	LE	SEM	G
Nitrogen Balance (g/d/$BW^{0.75}$)								
Nitrogen intake	1.56	1.74	0.05	0.03	1.55	1.66	0.06	0.21
Feces nitrogen	0.13	0.21	0.02	0.01	0.15	0.19	0.02	0.14
Urine nitrogen	0.46	0.54	0.06	0.33	0.47	0.47	0.05	0.97
Retained nitrogen	0.97	0.99	0.07	0.90	0.93	0.99	0.07	0.50

[1]RFI = Residual feed intake

[2]RIG = Residual intake and gain

[3]HE = High efficiency

[4]LE = Low efficiency

[5]G = Main effect of group

3.4.5. Gas exchange and HP – RFI

No differences in gas exchange (VO_2, VCO_2, and VCH_4) were observed between the HE and LE RFI groups (Table 6). In addition, no differences in HP ($kJ/BW^{0.75}$) were observed between the groups.

Table 6. Gas exchange and heat production in pre-weaning calves (55 ± 6 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI and RIG

Item	RFI[1]			*P*-value	RIG[2]			*P*-value
	HE[3]	LE[4]	SEM	G[5]	HE	LE	SEM	G
VO_2[6] ($L/day/kg^{0.75}$)	29.0	30.3	0.82	0.27	29.0	30.2	0.67	0.24
VO_2 (L/day)	568	567	15.2	0.95	559	554	11.0	0.75
VCO_2[7] ($L/day/kg^{0.75}$)	27.3	28.0	0.57	0.39	27.4	27.9	0.49	0.50
VCO_2 (L/day)	534	525	13.2	0.59	529	513	10.8	0.32
VCH_4[8] ($L/day/kg^{0.75}$)	0.04	0.06	0.02	0.37	0.05	0.03	0.02	0.45
VCH_4 (L/day)	0.67	1.21	0.42	0.35	0.89	0.58	0.31	0.47
VCH_4 (L/ kg of DMI of TSD[9])	0.93	1.77	0.56	0.30	1.27	0.93	0.45	0.60
HP[10] ($kJ/ kg^{0.75}$)	586	628	16.7	0.23	586	628	12.6	0.25
HP (kJ/d)	1184	1175	293	0.86	1163	1151	209	0.61

[1]RFI = Residual feed intake

[2]RIG = Residual intake and gain

[3]HE = High efficiency

[4]LE = Low efficiency

[5]G = Main effect of group

[6]VO_2 = Oxygen volume

[7]VCO_2 = Carbon dioxide volume

[8]VCH_4 = Methane volume

[9]TSD = Total solid diet

[10]HP = Heat production

3.4.6. Intake and performance - RIG

There was a tendency for greater solid intake (P = 0.07) and total intake (P = 0.08) in the HE RIG group compared to the LE RIG group. Moreover, an interaction was identified between treatment and week for milk intake (Table 2, Fig 2). The HE RIG group exhibited greater milk intake in the 5th week (P = 0.02). HE RIG animals had greater ADG and BW during the study period. Notably, there was a treatment x week interaction for heart girth (Table 2, Fig 2). Differences in heart girth were observed between week 5 to week 11, except in weeks 6 and 8 (P = 0.015; P = 0.07, respectively). HE RIG animals had lesser hip width during the study period, though no differences were observed for withers height and hip height.

3.4.7. Digestibility - RIG

No differences in DM, OM, CP, and NDF digestibility were identified. HE RIG animals tended to have greater EE digestibility (P = 0.07) when compared to LE RIG animals (Table 3).

3.4.8. Energy intake, losses, and energy use efficiency - RIG

No differences in energy intake (kJ/d/BW$^{0.75}$) and energy losses (% GE) by feces, urine, and heat were identified between HE and LE RIG animals. HE RIG animals had greater losses (% GE) by methane emissions, though no differences in energy use efficiency were found (Table 4).

3.4.9. Nitrogen balance – RIG

No differences in nitrogen balance (g/d/BW$^{0.75}$) between LE and HE RIG animals were observed (Table 5).

3.4.10. Gas exchange and heat production – RIG

No differences were observed in gas exchange (VO_2, VCO_2, and VCH_4) between HE and LE RIG groups (Table 6). In addition, no differences in HP (Kcal/BW$^{0.75}$) were identified.

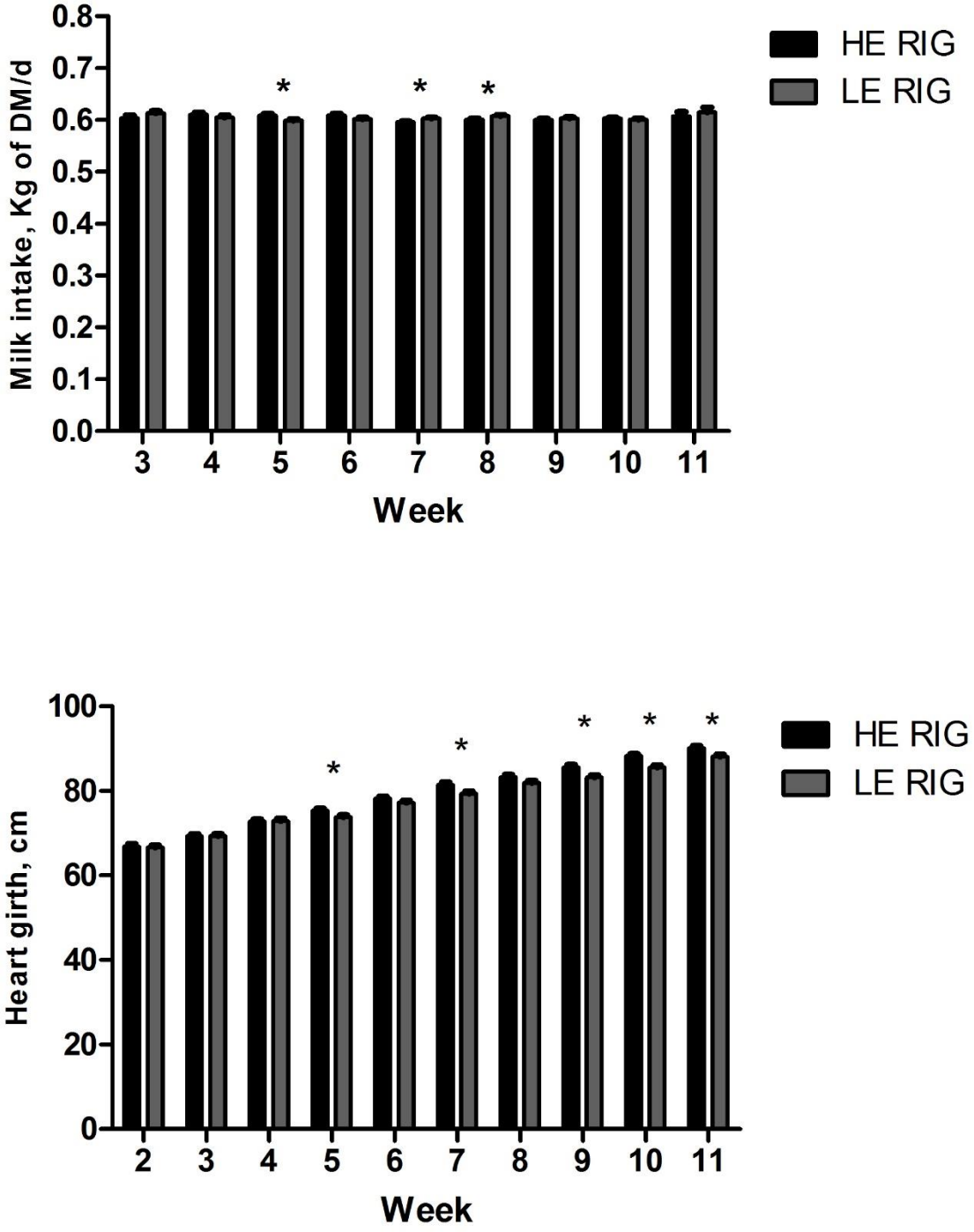

Fig 2. Weekly milk intake and heart girth in pre-weaning calves (14 to 77 days old) classified as high efficiency (HE) and low efficiency (LE) for RIG

3.5. DISCUSSION

3.5.1. RFI

FE divergence tests are applicable to pre-weaning calves, as divergence in RFI and RG were observed during the rearing phase (16). Animals classified as HE and LE for RFI had differences in TSD intake, which were related to differences in starter intake. Since milk is normally fixed in dairy systems, we decide to use this system during the evaluation. Milk supply was standardized in relation to metabolic weight at birth to reduce the chance of giving different amount of nutrients between the animals. Despite this, there was a tendency towards greater milk intake in HE RFI animals, which can be attributed to differences in BW between HE and LE RFI animals over the experiment. Because milk supply was based on metabolic weight, differences in body weight may have influenced milk intake tendencies.

The total difference in intake was 8.9% between the HE and LE groups for RFI, which was lesser than that observed by previous study [16] which reported a difference of 13% for crossbreed heifers (Holstein x Gyr) during the pre-weaning phase. Starter intake during the entire trial was also lesser than observed in previous trials conducted by our research team [7,16,17]. Differences in frame size (BW at birth: 25.2 ± 3.2 kg [mean ± SD]) may have influenced these results. Although the absolute values of DMI were lesser than those reported in previous studies, when data is viewed as % of BW, Gyr calves intake is similar to crossbred and pure Holstein calves [7,16,17].

Treatment x week interactions for TSD and starter intake indicated differences in intake between HE and LE RFI groups from the third week of age, when solid diet intake in pre-weaning heifers becomes expressive [18]. Results observed in the present trial suggests that variation in DMI are observed because pre-weaning phase. Recent study, reviewing biological factors related to RFI, showed that differences in intake behavior often explains part of the differences, but factors affecting differences in feeding behavior are not yet fully understood

[19].In addition, previous experiment evaluated the within-animal repeatability of intake, growth, and FE in different feed conditions (pasture and confinement) and concluded that DM intake, and to a lesser extent RFI, were somewhat repeatable traits [20]. Feeding behavior is determined by the integration of central and peripheral signals in brain feeding centers [21]. Notably, a study that evaluated hypothalamic metabolomic profiling in cattle with divergent RFI suggested that there are differences between HE and LE groups that may be related to differences in the central regulation of intake[22]. Differences in meal patterning appears to be related to differences in RFI during pre-weaning phase.

There were no differences in water intake between the HE and LE groups for RFI despite its positive relationship with DMI [16,23]. The water intake observed in the present study was lesser than that observed by previous studies [16,17]. This could potentially be related to the season, because the present study was conducted during the colder autumn and winter, when water intake becomes lesser. Such differences among studies can also be related to the animal's breed, because we are unaware of any study that evaluated water intake in pre-weaning Gyr heifer calves.

There were no differences in body measures, except a tendency for greater hip width in LE RFI heifers. Little is known about the relationship between RFI and body measurements. Most studies on growing animals have been performed in beef cattle, and no differences were observed in body structure between the HE and LE groups for RFI [24-27,28]. In addition, a study observed that the phenotypic correlation between RFI and ADG or body size was close to zero, indicating that selection based on RFI would not affect growth or body size [27].

HE RFI animals exhibited increased CP and EE digestibility and tended to have improved DM and OM digestibility. Pre-weaning dairy calves ingest a high protein and fat diet with a low proportion of fiber, which may explain why we did not observe differences in NDF digestibility.

It is known that as level of feed intake relative to maintenance increases, the digestion of feed tends to decrease. However, over and above systematic variation due to the amount of feed eaten, there is also genetic variation in the total tract digestion of feed [5].

Previous studies on beef steers [29], and lactating dairy cows [30] have demonstrated that animals classified as HE RFI have improved digestibility. Experiment also determined that nutrient digestibility was moderately repeatable across different diets [30]. Research conducted on pigs selected for RFI noted greater digestibility in HE RFI animals, and hypothesized that such findings could be related to the increased activity of intestinal microbial populations associated with the greater gene expression levels of intestinal nutrient transporters [31,32]. We suggest that greater digestibility found in pre-weaning heifers can also be related to greater intestinal activity, because the intestinal contribution to total tract digestion during this growth phase is high; however, more studies should be made to confirm this hypothesis.

Positive correlations were observed between RFI and GEI (r = 0.502; P = 0.028), indicating that LE RFI had a greater gross energy intake. Despite this, energy losses in feces were also positive correlated with RFI (r = 0.41; P = 0.08). Overall, while LE RFI animals eat more, they have greater fecal losses due to lesser digestibility, which results in similar DEI.

Positive correlations were also found between RFI and nitrogen intake (r = 0.50; P = 0.03) and losses in feces (r = 0.6; P = 0.005). This can be attributed to the differences in CP digestibility, because LE RFI animals had a greater intake but lowest CP digestibility, resulting in similar retained nitrogen values when compared with HE RFI group. In terms of energy intake, GEI was greater in the LE RFI group, but digestible and metabolizable intake were the same between HE and LE animals. This can be attribute to greater digestibility in the HE RFI group. When we evaluate the losses (% GE), a tendency was found to greater fecal losses in LE RFI animals; however, no differences in methane losses (% GE) and methane production parameters between HE and LE RFI were found among pre-weaning heifers in this trial. No

differences in HP (kJ/kg$^{0.75}$ and kJ/d) between the groups were observed, although heat losses (% GE) were greater for HE RFI animals. Overall, the efficiency of using metabolizable energy was the same between HE and LE RIF animals, which is evident from the HP:ME and EB:ME energy ratio (Table 4).

A previous study, that used a face mask method to evaluate methane production in pre-weaning heifers [16] did not observe significant methane production during this phase. Pre-weaned heifers do not have a fully developed rumen and also eat a grain-heavy diet that makes methane production a minor source of energy loss in these animals. Additionally, even in animals with a developed rumen, lesser methane production is not always observed in HE RFI animals. The reduction of feeding level generally increases the mean retention time of digesta in the rumen [33], which can increase methane production. Moreover, HE RFI animals often have greater total tract DM digestibility, which implies a greater amount of substrate available for fermentation and methanogenesis per unit feed [34].

3.5.2. RIG

The use of RFI as an FE index can rank slow-growing animals such as HE RFI [4]. This is not applicable for the dairy industry due to the fact that slow-growing animals may take longer to achieve puberty and thus have a greater age at first calving.

A previous study proposed the use of RIG as an alternative index [4] because it can identify animals that grow rapidly and eat proportionally less than expected. The observed differences in intake and performance between animals classified as HE RIG and LE RIG in this trial met the purpose for the index, because HE RIG animals exhibited greater ADG and BW during the efficiency test. HE RIG animals also had greater heart girth, which was somewhat expected because heart girth has a high correlation with BW [35] HE RIG animals also seem to have narrow hip width compared to LE RIG animals.

Differences in body measurements such as hip width can indicate differences in phenotypic and functional features in animals [36]. A previous study noted a positive correlation between hip width and energy-corrected milk and a negative correlation between hip width and productive life, which suggests that narrow animals are more prone to be less productive, and thus more likely to be culled. Thus, the impacts of RIG utilization for selecting productive animals must be further investigated.

Interaction between treatment x week was observed for milk intake between RIG groups. Differences in milk intake between the groups are result of slightly fluctuations in milk intake in individual animals. In week 5 the animals were subjected to a dehorn process. Although we adopted practices to reduce the stress associated to dehorn procedure, individuals can have different responses to stress, which affected the milk intake in some animals. Because the present experiment is an efficiency assay, the real intake of the animals over the 63 days were computed.

No differences in DM, OM, CP, and NDF digestibility were observed, although HE RIG animals tended to have greater EE digestibility when compared to LE RIG animals. Greater efficiency in fat absorption can partially explain the greater ADG of these animals, because fat is an energy-dense nutrient.

No differences were found in energy intake, energy losses, and energy use efficiency, nitrogen balance, gas exchange, and HP between HE and LE RIG animals, with the exception of high methane loss (% GE) in HE RIG animals.

As previously discussed, the methane emissions in pre-weaning phase is low. In addition, the variation in methane production between animals was high during the study period. Due to the high variability and consequently high SE, there were no differences in daily methane production—even when considering the metabolic weight and intake of the animals.

The use of methane units as a percentage of losses normalized the data distribution and made it possible to observe the difference in methane production between the groups, with losses being proportionally greater for the HE RIG group. In the present study, the greater methane losses in the HE RIG group may have been related to the quantity of substrate available for fermentation and methanogenesis (35).

3.6. FINAL CONSIDERATIONS

The use of RFI and RIG indexes resulted in groups with different characteristics and it should be taken into account in future research and animals selection programs. Digestibility of DM, OM, CP, and EE were factors that most heavily impacted differences in RFI between pre-weaning calves. Despite the lesser intake of GE and nitrogen, RFI animals had the same energy and nitrogen retention among groups, which resulted in the same ADG. Then, intake had greater weight when grouping the calves by RFI. However, when grouping by RIG the divergency was more associated to body measurements, which may be related to differences in the composition of gain. Future research should evaluate how the use of different indexes would impact in breeding programs and which of these calves' characteristics are associated to its efficiency indexes when cows.

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4. CAPÍTULO IV – ARTIGO II

Relationship between feed efficiency indexes and thermography, blood and ruminal parameters in pre-weaning dairy heifers

4.1.ABSTRACT

The objective of this study was to evaluate whether pre-weaning heifer calves divergent for residual feed intake (**RFI**) or residual feed intake and body weight gain (**RIG**) exhibit differences in thermography, blood and ruminal parameters. Gyr heifer calves 32 were enrolled in a 63-d trial and classified into 2 feed efficiency (**FE**) groups based on RFI and RIG (mean ± 0.5 SD). The groups were classified as high efficiency (**HE**) RFI (HE RFI, n = 9; HE RIG, n = 10), and low efficiency (**LE**) RFI (LE RFI, n = 10; LE RIG, n = 11). HE and LE calves had RFI values of - 0.052 and 0.049 kg/d ($P < 0.05$), respectively. At birth, the amount of whole milk provided for each animal was calculated based on their metabolic weight at birth (42% x $BW^{0.75}$). The liquid diet was divided into two meals at 0700 and 1400. The total solid diet (**TSD**) was composed of 92% concentrate and 8% of *tifton* 85 hay chopped in 5 cm length as-fed. Intake was measured daily. Blood concentrations of insulin, BHB, urea and glucose, and pH, $N\text{-}NH_3$ and volatile fatty acids (**VFA**) in ruminal fluid were evaluated at 14, 28, 42, 56 and 70 days of age, respectively. Thermal images of the calves were taken with an infrared camera on d 56 (±3) at 0600 h, before the morning feeding. The HE RFI group consumed 8.9% less solid diet than the LE RFI group. Total VFA concentration (µmol/mL), proportion of acetate (%VFA) and propionate (%VFA) in ruminal fluid were greater for LE than HE RFI heifers. Blood urea concentration tended to be higher in LE than HE RFI heifers. HE RIG animals tended to have greater ruminal proportion of acetate (%VFA) and lower propionate (%VFA). Blood insulin concentration was greater and blood glucose tended to be greater for LE than HE RIG animals. LE RIG had higher left rib, left flank and anus surface temperature measured by infrared

thermography in comparison to HE RIG group. Differences in ruminal fermentation does not seem responsible for the higher efficiency in pre-weaning heifers while differences in protein metabolism seems to affect RFI during this phase. Differences in insulin and glucose may play a whole in RIG and can be related to differences in body composition. Infrared thermography seems to be correlated to RIG but not to heat production (**HP**) in pre-weaning calves.

Keywords: residual feed intake, residual intake and body weight gain, ruminal parameters, blood metabolites

4.2.INTRODUCTION

In milk production systems feed corresponds to 50% of the total cost of production (1), and therefore, feed efficiency (**FE**) is essential to increase profitability (2). FE indexes as a future tool to select more efficient animals have been widely studied, and residual feed intake (**RFI**) is a FE index calculated as the difference between actual and expected animal feed intake, at a certain level of production (3).

The independence of RFI from productive parameters led researchers to suggest that this measurement describes the inherent variation of basic metabolic processes, mainly related to intake and digestion, anabolism and catabolism, activity and thermoregulation processes (4).

Understanding physiological basis of divergence between high and low FE cows is important in identifying and selecting more efficient animals without compromising other physiological processes. In addition, it can help develop FE markers, reducing the cost of identification and selection of efficient cows in future studies (5).

Some studies linked RFI during lactation with RFI during the growth phase indicating that RFI may be a lifetime trait (6,7). Studying RFI in the early phases of life can help reduce the cost of evaluations and increase the progress on understanding these traits. Most of the studies in growing cattle are executed during the post weaning phase.

Selection of slow-growing animals can be a problem associated with RFI (8) and residual intake and body weight gain (**RIG**) was proposed as an alternative index to select efficient growing animals. There is no research in literature evaluating the use of RIG as FE index in pre-weaning heifers and its effect on physiological and productive parameters in comparison to RFI.

The objective of this study was to evaluate whether pre-weaning heifer calves divergent for FE indexes exhibit differences in thermography, blood and ruminal parameters. Our hypothesis is that there are differences in the metabolic processes of the heifers that justify the divergence in feed efficiency indexes.

4.3.MATERIAL AND METHODS

4.3.1. Calves, Housing, Management, and Treatments

Data presented in this paper is part of experiment that classified animals into high and low feed efficiency groups using two feed efficiency indexes (RFI and and RIG). Detailed descriptions of methodology to classify the animals and performance data is presented in the companion paper (Lage et al.,2019). A brief overview of methods associated with RFI and RIG determination and performance data in the heifers are presented here. A total of 32 Gyr heifer calves (body weight at birth = 25.2 ± 3.2 kg) produced by in vitro fertilization and born during the autumn (April to June) were used.

After birth, the animals were immediately separated from their dams, weighed, and had the umbilical cord immersed in iodine solution (10%). Colostrum was administered (10% of BW; >50g of IgG/L) up to 6 h after birth. Colostrum was evaluated using an electronic refractometer (Palm Abbe PA203x, Misco, Cleveland, Ohio, USA). Blood samples were collected via jugular venipuncture up to 48 h post-birth to measure total plasma protein (g/dL) using an electronic refractometer (Palm Abbe PA203x, Misco, Cleveland, Ohio, USA).

The amount of milk offered for each heifer was based on their metabolic weight at birth. The amount of milk routinely supplied in Brazilian farms, which is 6 L of whole milk for calves with a mean birth weight of 35 kg (9), was used as a reference. This equates to 42% of the metabolic weight at birth in liters of milk. The objective was to standardize the amount of nutrients supplied to the heifers from the liquid diet. The mean weight of the heifers at birth was 25.2 ± 3.2 kg (mean ± standard deviation (**SD**)). Consequently, the daily milk supply was 4.70 ± 0.46 L.

Heifers received transition milk until 3 days of age, and whole milk from the 4th to the 77th day of age. On the 78th day of age, milk supply was reduced by half and the calves were weaned on the 81st day of age. During the pre-weaning period, heifers received a liquid diet divided into 2 equal meals offered at 0700 and 1400 h in nipple buckets (Milk Bar®, New Zealand).

Water and total solid diet (**TSD**; hay and starter) were offered *ad libitum* in buckets (10% of refusals for solid diet). The solid diet was composed of 92% starter (Soymax Rumen pre-initial, 18% flocculated, Total Alimentos, Três Corações, Minas Gerais, Brazil) and 8% Tifton 85 (*Cynodon* spp) hay chopped in 5 cm length, as fed. Samples of the TSD were collected 3 times a week and composited and homogenized weekly. Individual refusals were collected daily and were composited weekly. Samples were stored at -20°C until processing. Feed samples were oven dried at 55 °C for 72 hours, ground through a 1 mm sieve in a Wiley type mill (model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) and analyzed for dry matter (**DM**), crude protein (**CP**), ether extract (**EE**), ash (10) and neutral detergent fiber (**NDF**) (11). Gross energy was determined using an adiabatic calorimeter (IKA - C5000, IKA® Works, Staufen, Germany).

The analyzed composition of TSD was as follows (DM basis): 84% DM, 78.5% OM, 18.5% CP, 32.5% NDF, 3.33% ether extract and estimated gross energy was 4.235 kcal/kg of DM (Lage et al., 2019).

Milk, TSD, and water intake were measured individually. Daily intakes of milk, TSD and water were calculated by the difference between offers and the refusals. Feed and water were weighed using a bench scale (9094 plus, Toledo®, São Bernardo do Campo, São Paulo, Brazil) and a portable scale (WH-A04, WeiHeng, China), respectively.

Milk samples were collected twice daily (morning and afternoon) and analyzed for total solids, CP, lactose, and fat. Milk component analysis was performed using an infrared analyzer (Bentley model 2000, Bentley Instruments Inc., Chaska, MN, USA). Mean ± SD values for the milk analysis were: 12.9% ± 1.1 for total solids, 4.4% ± 1.01 for fat, 3.1% ± 0.1 for CP, and 4.5% ± 0.1 for lactose.

Body weight was recorded before the morning meal on days 3 and 7 after birth, and weekly from day 8 onward.

Intake and performance were evaluated from the 14^{th} to the 77^{th} day of age, and the indexes were calculated based on 63 days of observation (12). The growth rate of the heifers was modeled by linear regression of BW against time over the trial duration, and the regression coefficients were calculated for the average daily gain (**ADG**) of each animal. Mean daily feed intake was calculated for each animal over the trial period and corrected for DM. The average metabolic weight ($\mathbf{BW^{0.75}}$) was calculated using the BW at the 46^{th} day of age, which was the middle of the test period.

Dry matter intake, $BW^{0.75}$, and ADG were used to estimate RFI and residual body weight gain using linear regressions (3) and details of the calculation is presented in Lage et al. (2019).

Based on these indexes, the heifer calves were classified into four groups: high efficiency (**HE**) and low efficiency (**LE**) for RFI and RIG. HE indicated RFI < 0.5 SD below

the mean (n = 9) and RIG > 0.5 SD above the mean (n = 10), while LE indicated RFI > 0.5 SD above the mean (n=10) and RIG < 0.5 SD below the mean (n = 11). The remaining calves were classified as intermediary (n = 13 [RFI] and n = 11 [RIG]).

4.3.2. Blood collection and analysis

To determine the concentration of BHB, urea and glucose, blood samples were taken at 14, 28, 42, 56 and 70 days of age and to determine the concentration of insulin blood samples were taken at 28, 42, 56 and 70 days of age. All blood samples were taken via venipuncture of the jugular vein, 3 h after the morning meal. Tubes without anticoagulant for insulin, BHB and urea and with fluoride EDTA for glucose (Vacutainer; Becton, Dickinson and Company) were used. The tubes were stored on ice until centrifugation at 1,800 x g for 10 min at room temperature (22-25 °C).

Aliquots of serum and plasma were stored at -20 °C until further analysis. Glucose and urea were measured using a colorimetric enzymatic method (Kovalent do Brasil Ltda., Rio de Janeiro, Brazil). Insulin was analyzed using a Bovine Elisa kit (Bovine Insulin ELISA, Kit No. 10-1201-01, Mercodia AB, Uppsala, Sweden). The coefficients of inter- and intra-assay variation were 11.2% and 9.1%, respectively. BHB determination was performed using an enzyme kinetic kit (RANBUT kit - Ref.: RB 1007; RANDOX Laboratories - Life Sciences Ltd, Crumlin, UK). All the readings were performed on an EON microplate spectrophotometer (Biotek Instruments Inc., Winooski, VT).

4.3.3. Rumen variables and analysis

Ruminal fluid samples were collected at 14, 28, 42, 56 and 70 day of age using an esophageal tube, 3 h after morning feeding. The liquid was double filtered through cheesecloth and pH was measured (Mettler Toledo®, Columbus, Ohio, United States).

For N-NH_3 determination, 5 mL of filtered ruminal liquid was acidified with 1 mL of sulfuric acid (500 mL/L) and stored at -20°C until further analysis. Analysis were performed

after distillation of Kjeldahl with magnesium oxide and calcium chloride according to the method 920.03 (10).

For volatile fatty acids (**VFA**) determination, 1 mL of 20% metaphosphoric acid was added to 10 mL of filtered ruminal liquid and stored at -20°C. Samples were defrosted at room temperature (22-25°C) and centrifuged at 13,000 rpm for 10 minutes. The samples were analyzed by high performance liquid chromatography (Waters Alliance and 2695 Chromatograph, Waters Technologies of Brazil LTDA, Barueri, São Paulo, Brazil).

4.3.4. Infrared Thermography (IRT)

Thermal images of the calves were taken with an infrared camera (FLIR T420, FLIR Systems Inc., Wilsonville, OR) on day 56 (±3) at 0600, before the morning feeding. The following standards were established for imaging: 0.5 m distance between the thermograph and the evaluated anatomical region, reflectance temperature of 20°C, and emission value of 0.98, according to the manufacturer's recommendations for biological tissues and to values used by (13). All thermal images were obtained in a roofed area. The evaluated anatomical regions were eye, jaw, muzzle, left-side ribs, left-side flank, right front limb, anus and vulva.

Calves were manually restrained during the evaluations, with no manipulation of the evaluated areas. The average ambient temperatures recorded during imaging evaluations ranged between 13.8ºC and 20.8°C (relative humidity between 89% and 99%). The generated images were processed and interpreted using the FLIR Tools 5.6 software (FLIR Systems Inc.).

To establish a constant area of evaluation, a figure was drawn on the image surface and it was then dragged to the region of the skin located in the chosen area (14). Only the maximum temperature within each delimited area was considered, to reduce the interference of factors such as contamination by water, feces, urine, or contact with colder surfaces, which could influence the thermogram. The rectal temperature of each animal was measured immediately after the IRT evaluations using a digital thermometer (TH198, GTech, Rio de Janeiro, Brazil).

4.3.5. Statistical analyses

The statistical analysis was performed using the SAS software (SAS Institute Inc., Cary, NC, version 9.4). To evaluate the effects of efficiency in the groups, the MIXED procedure was used, according to the model:

$$Y_{ijk} = \beta_0 + \beta_1 A_{ij} + \beta_2 B_{ij} + G_i + M_k + GM_{ik} + \delta_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, β_0 is the intercept, $\beta_1 A_{ij}$ is the regression coefficient for the covariate initial BW; $\beta_2 B_{ij}$ is the regression coefficient for the covariate total serum protein; G_i is the fixed effect of efficiency group (RFI or RIG); M_k is the fixed effect of repeated measure (day or week); GM_{ik} is the fixed effect of interaction between group and repeated measure; δ_{ij} is the random error between animals within treatment and ε_{ijk} is the random error between measurements within animals. The best covariance structure for repeated measures was chosen by the lower corrected Akaike information criteria (**AICc**). For significative interaction between group and repeated measure the differences among groups within measures were evaluated using the SLICE statement. Significance of the effects was declared at $P \leq 0.05$ and tendency was accepted when $0.05 \leq P \leq 0.10$.

4.4.RESULTS

4.4.1. RFI

Detailed information about feed efficiency classification and performance data is presented in the companion paper (Lage et al., 2019). In brief, the average RFI for the HE group was -0.052 kg/d and for the LE group was 0.049 kg of DMI/d (SEM = 0.010, $P < 0.01$). HE RFI group consumed 8.9% less than LE RFI group. Total intake was 0.76 and 0.84 kg/d for HE and LE RFI groups, respectively (SEM = 0.02, $P = 0.002$). Average daily gain was 0.60 and 0.59 kg/d for HE and LE RFI groups, respectively (SEM = 0.02, *P*-value = 0.67) and body

weight was 48.2 and 46.6 for HE and LE RFI groups, respectively (SEM = 0.77, *P* = 0.17) (Lage et al.,2019).

No statistical differences (*P* > 0.17) were observed for ruminal pH, ammonia concentration (%) and proportion of butyrate (%VFA) between HE and LE RIF groups (Table 1). Total VFA concentration (µmol/mL) in ruminal fluid was greater (*P* = 0.001) for LE than HE RFI heifers. In addition, propionate (%VFA) was greater in LE RFI group (*P* = 0.002) while acetate (% VFA) was lower for this group (*P* = 0.01).

Tabela 1. Parametros ruminais em bezerras durante a fase de aleitamento (14 a 77 dias de idade) classificados como alta eficiências (AE) e baixa eficiência (BE) para CAR e CGR

Item	RFI[1]		SEM	*P*-value	RIG		SEM	*P*-value
	HE	LE			HE	LE		
Ammonia N, %	14.7	15.6	0.71	0.31	16.7	16.2	0.905	0.67
pH	6.52	6.31	0.109	0.17	6.39	6.36	0.089	0.79
Total VFA, µmol/mL	32.9[b]	43.4[a]	1.99	0.001	34.6	39.8	2.271	0.11
VFA, % of total VFA								
Acetate	0.75[a]	0.67[b]	0.020	0.01	0.74	0.69	0.019	0.08
Butyrate	0.04	0.06	0.008	0.14	0.05	0.05	0.006	0.75
Propionate	0.21[b]	0.27[a]	0.013	0.002	0.22	0.26	0.015	0.06

[a,b]Means with different letter superscripts differ at P ≤ 0.05.

[1]RFI = Residual feed intake.

[2]RIG = Residual intake and body weight gain

[3]HE = High efficiency

[4]LE = Low efficiency

[5]Main effect of group; Treatment × week interactions were, *P* ≥ 0.10, except total VFA (µmol/mL) (*P* = 0.017), acetate (%VFA) (*P* = 0.034) and propionate (%VFA) (*P* = 0.015) for RFI groups and *P* ≥ 0.11 for all variables in RIG groups.

[6]Standart error of the means

Interaction treatment × week was observed for total VFA concentration (*P* = 0.017). LE RFI heifers had higher ruminal VFA concentration at 28 and 42 d of age in comparison to HE

RFI heifers (Table 1, figure 1). Interactions treatment × week were also observed for acetate and propionate (%, $P = 0.034$; $P = 0.015$, respectively). Acetate was lower in LE RFI group at 28 and 42 d of age while propionate was greater in LE than HE RFI group at 28, 42 and 56 d of age (Table 1, figure 1).

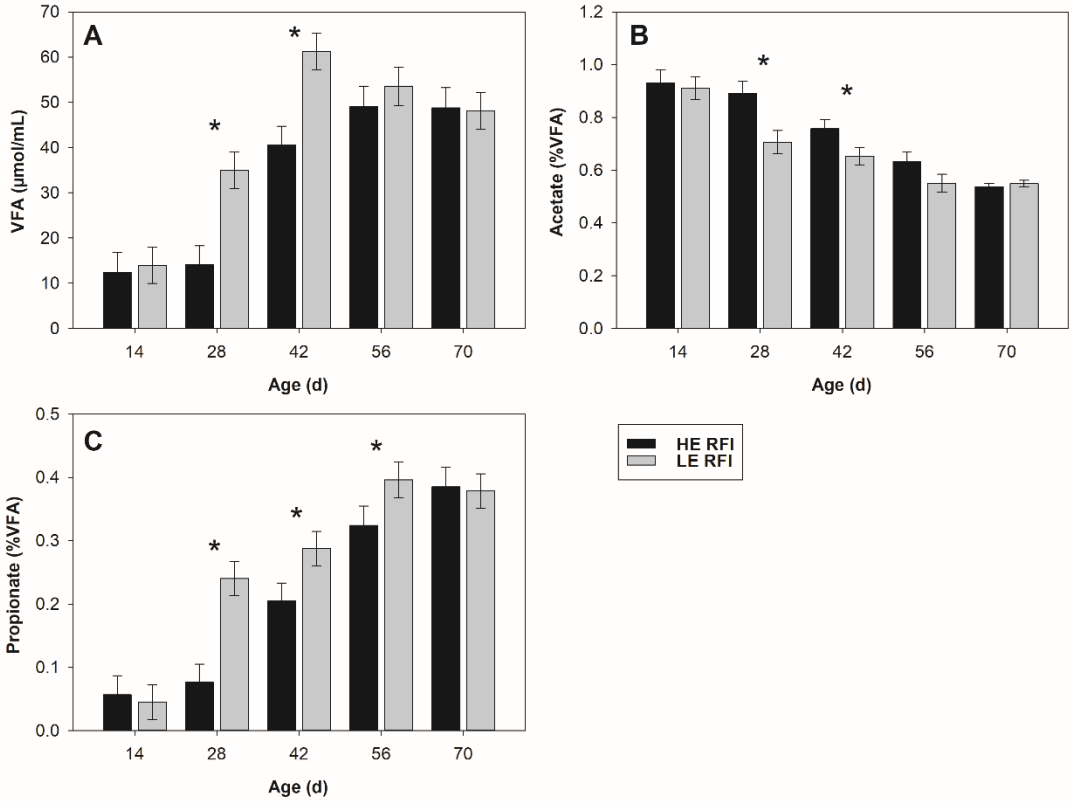

Figure 1. (A) VFA (µmol/mL), (B) acetate (% VFA) and propionate (% VFA) in ruminal fluid of animals classified as high efficiency (HE) and low efficiency (LE) of residual feed intake (RFI) from the 3rd to the 11th week of age. Asterisk (*) represents the existence of difference between treatments ($P < 0.05$). Error bars represent standard error of the mean (SEM).

Blood BHB, glucose and insulin concentrations did not differ between RFI groups ($P > 0.22$) (Table 2). Blood urea concentration tended ($P = 0.06$) to be higher in LE than HE RFI heifers.

Table 2. Blood parameters in pre-weaning calves (14 to 77 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI and RIG

Item	RFI[1]		SEM	*P*-value[5]	RIG[2]		SEM[6]	*P*-value
	HE[3]	LE[4]			HE	LE		
BHB, µg/L	0.51	0.58	0.038	0.22	0.53	0.57	0.037	0.52
Glucose, mg/dL	125	123	6.88	0.84	118	125	2.72	0.07
Insulin, pmol/L	2.50	2.75	0.185	0.34	2.47	3.31	0.23	0.01
Urea, mg/dL	18.4	21.4	1.02	0.06	19.6	21.2	0.74	0.12

[1]Residual feed intake

[2]Residual intake and gain

[3]High efficiency

[4]Low efficiency

[5]Main effect of group; Treatment × week interactions were, $P \geq 0.21$ for RFI groups and $P \geq 0.48$, except blood insulin concentration ($P = 0.001$) for RIG groups.

[6]Standart error of the means

Skin surface temperature measured by infrared temperature did not differ between RFI groups but moderate correlation (0.47, $P = 0.048$) between flank temperature and RFI was observed (Table 3 and 4).

Table 3. Skin surface temperature measure by infrared thermography in pre-weaning calves (56 days old) classified as high efficiency (HE) and low efficiency (LE) for RFI and RIG

Item	RFI[1]		SEM	*P*-value[5]	RIG[2]		SEM[6]	*P*-value
	HE[3]	LE[4]			HE	LE		
Temperature (ºC)								
Anus	37.7	38.2	0.24	0.13	37.4	38.2	0.24	0.03
Left eye	36.2	36.2	0.35	0.99	35.9	36.3	0.24	0.32
Left flank	30.8	32.4	0.82	0.18	30.8	30.9	0.63	0.03
Left foot	26.4	24.5	1.19	0.28	25.3	24.2	1.11	0.50
Left jaw	32.6	33.1	0.39	0.43	31.6	32.7	0.53	0.17
Left rib	32.7	33.1	0.52	0.58	32.1	33.6	0.42	0.02
Muzzle	22.8	22.5	0.79	0.74	22.6	22.3	0.75	0.80
Rectal	38.3	38.3	0.14	0.89	38.3	38.4	0.15	0.15
Vulva	36.2	36.7	0.29	0.23	36.2	36.7	0.22	0.15

[1]Residual feed intake

[2]Residual intake and gain

[3]High efficiency

[4]Low efficiency

[5]Main effect of group

[6]Standart error of the means

Table 4. Correlations between feed efficiency indexes (RFI and RIG) and energy intake, losses and energy use efficiency, nitrogen balance and skin surface temperature

Item	RFI[1]		RIG[2]	
	R	*P*-value[3]	R	*P*-value
Anus temperature	0.33	0.166	-0.339	0.132
Eye temperature	0.106	0.665	-0.132	0.567
Flank temperature	0.47	0.048	-0.594	0.005
Foot temperature	-0.305	0.217	0.329	0.155
Jaw temperature	0.342	0.151	-0.272	0.231
Muzzle temperature	-0.048	0.844	0.13	0.573
Rectal temperature	0.01	0.965	-0.06	0.794
Rib temperature	0.301	0.222	-0.446	0.048
Vulva temperature	0.244	0.313	-0.183	0.424

[1]Residual feed intake

[2]Residual intake and gain

[3]Main effect of group

4.4.2. RIG

The average RIG for the HE group was 0.080 kg/d and for the LE group was -0.077 kg/d. HE RIG group had an ADG of 8.6% higher than the LE RIG group (Table 2). Total intake was 0.78 and 0.80 kg/d for HE and LE RFI groups, respectively (SEM = 0.010, *P* = 0.08). Average daily gain was 0.60 and 0.55 kg/d for HE and LE RFI groups, respectively (SEM = 0.02, *P*-value = 0.04) and body weight was 47.8 and 46.3 for HE and LE RFI groups, respectively (SEM = 0.25, *P* value = < 0.001) (Lage et al., 2019).

No differences in ruminal parameters between HE and LE RIG groups were observed (Table 1), except a tendency to greater ruminal proportion of acetate (%VFA) (*P* = 0.08) and lower proportion of propionate (%VFA) (*P* = 0.06) concentrations in HE RIG in comparison to LE RIG heifers.

Blood insulin concentration was greater (*P* = 0.02) for LE than HE RIG animals (Table 2). Blood glucose concentration also tended (*P* = 0.06) to be higher in LE RIG groups but no differences in BHB and urea between RIG groups were observed. Treatment × week interaction for blood insulin concentration was observed (*P* = 0.001). Insulin was greater in LE comparison to HE RIG animals at 56 days of age (Figure 2).

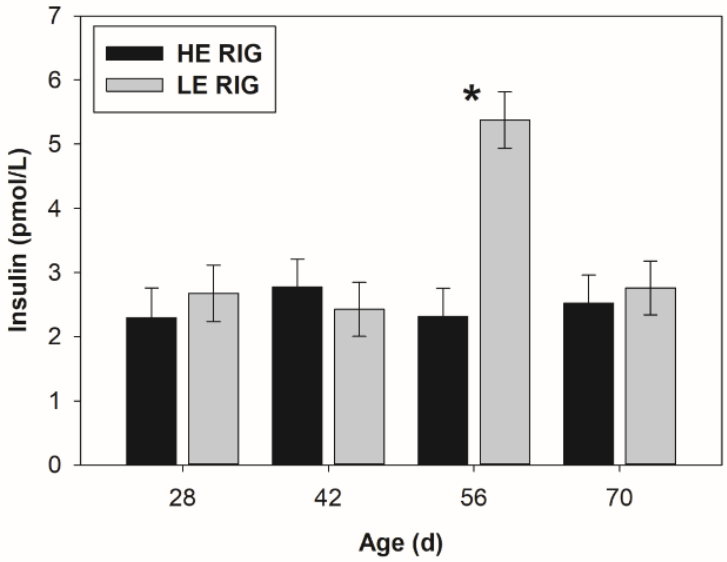

Figure 2. Blood insulin (pmol/L) concentration from 28 to 70 days of age in animals classified as HE or LE RIG. Asterisk (*) represents the existence of difference between treatments ($P < 0.05$). Error bars represent standard error of the mean (SEM)

LE RIG had higher ($P = 0.03$) left rib, left flank and anus surface temperature measured by infrared thermography in comparison to HE RIG group (Table 6). Hence, moderate correlations between flank (-0.594, $P = 0.005$) and rib temperature (-0.446, $P = 0.048$) with RIG were observed.

4.5.DISCUSSION

4.5.1. RFI

Residual feed intake is defined as the difference between observed, and expected feed intake based on the requirement to support both maintenance of BW and growth (16). HE RFI animals eat less than average to support certain level of production (3) and based on that, differences in intake between HE and LE RFI groups observed in this experiment were

expected. Detailed discussion about intake and performance data can be found in Lage et al. (2019).

No differences in pH and ammonia between RFI groups were observed. Other experiments also did not find differences in ruminal pH between the HE and LE groups for RFI (17,18), demonstrating same rumen environment conditions between RFI groups.

Differences in ruminal parameters between HE and LE RFI groups seem to be driven by differences in intake. LE RFI heifers had a higher solid intake followed by greater total VFA (µmol/mL) and proportion of propionate (% VFA). Furthermore, LE RFI had lower acetate (% VFA) when compared to HE RFI groups. A possible explanation for these results is that presence of fermentable substrate in the rumen determines the extent of VFA production. A greater consumption of solid diet leads to higher VFA concentration (19). Moreover, pre-weaning heifers diet has a high proportion of grain, which will favour greater propionate production (20).

Another factor that may influenced the lower acetate and higher propionate in LE RFI group is a higher passage rate due to higher intake (21). Shorter time of feed retention in the rumen can reduce the degradability of fibrous components, reducing the production of acetate in relation to propionate (22). A trial conducted with feedlot animals under high grain diet also observed higher propionate and lower acetate ruminal concentration for LE RFI animals (17).

Differences in ruminal colonization and in the efficiency in the utilization of energy in the rumen and post rumen can be responsible for the differences in efficiency between the groups. Different phylotypes of bacteria was observed in the rumen of steers classified as high and low RFI, and specific phylotypes of bacteria colonize preferably HE RFI animals (23). Presence of *Lachnospiraceae* in HE RFI was associated to high efficiency in animals classified by RFI (24). Individual microbiota variation is the result of an individual variation in genetics,

behaviour and environmental effects but the dynamics on how these factors influence feed efficiency remain unknown (25).

Ruminal concentration of VFA is an indicator of the metabolic activity of the microbial ecosystem, but do not directly reflect on how the animals utilize these products (26). Differences in post-absorptive efficiency of energy use related to activity, body composition and other metabolic processes previously reported in literature (16), can partially explain why the group with higher VFA concentration did not have a better performance.

In the present experiment, HE RFI animals had increased digestibility of CP and EE and tended to have improved total DM and organic matter (OM) digestibility (Lage et al., 2019). A possible reason that explains why the HE RFI animals kept the same ADG eating less and producing less VFA can be related to a more efficient digestion and absorption of nutrients in the intestine. Pre-weaning heifers depend largely on the intestinal digestion for the absorption of the nutrients and that appears to be the factor responsible for the RFI difference between the groups.

Blood concentration of metabolites and hormones can be a useful tool to understand differences in metabolism. Several RFI studies have evaluated blood parameters to understand differences in feed efficiency and tried to detect potential physiological markers for FE (27,28). In the present experiment, no differences in glucose, insulin and BHB were observed. This agrees with some studies performed in growing animals (5,29), while others have observed a tendency ($P < 0.10$) for higher insulin in LE RFI groups (30) or a weak correlation between higher insulin and higher RFI values (6).

In full developed ruminants, blood BHB is mostly related to the fatty acid oxidation originating from the mobilization of body fat, which is not important during pre-weaning phase (31). In young ruminants, the main contribution to plasma - concentration of butyrate is related to its production in the rumen (31,32). In the present study, no differences in ruminal butyrate

concentration (% VFA) between RFI groups were observed, which can explain the lack of differences in blood BHB.

LE RFI animals tended to have higher blood urea concentration in comparison to HE RFI animals. Association between high levels of blood urea and LE RFI have been previously reported in the literature (30,33). This is often related to greater protein intake (5). However, in the present experiment LE RFI animals had higher protein intake but same retained nitrogen (Lage et al., 2019), being differences in protein intake not the only reason regarding urea differences. Urea is a product of protein degradation (34) and a higher urea concentration in LE RFI heifers can be related to a greater AA catabolism in less efficient steers. There is evidence that LE RFI animals are more susceptible to stress and they are more prone to mobilize muscle from the tissue, which can contribute to the increase in blood urea concentration (35). More studies need to be done to better understand this mechanism in pre-weaning dairy heifers.

Variation in metabolism can affect heat production, which can account for up to 73% of the variation in RFI (4). Skin surface temperature, measured by thermography, can indirectly indicate differences in heat production (HP). Lower maintenance requirements in HE RFI animals can potentially result in less heat being dissipated through the body surface (13). While some studies reported higher correlations between HP and skin surface temperature in some body locations (36,37). Experiment conducted with pre-weaned dairy heifers, did not observed differences between skin surface temperature for RFI groups, despite of finding higher HP measured by face mask method in HE animals (38). In the present experiment, no differences in skin surface temperature measured by infrared thermography were observed between RFI groups. In line with that, no differences in HP between the groups were found (Lage et al., 2019). Flank temperature was moderately correlated with RFI. However, HP and infrared temperature were not correlated.

4.5.2. RIG

Residual feed intake and body weight gain was proposed to be an intermediary index between RFI and RG (8). Hence, the observed differences in intake and performance between the groups in this trial met the purpose for the index. Detailed discussion about performance and intake can be found in Lage et al. (2019).

As discussed above for RFI, changes in VFA concentration and percentage of each VFA followed the intake parameters. LE RIG animals tended to have a higher solid and total intake followed by a tendency to have higher proportion of propionate (%VFA) and lower proportion of acetate (%VFA) in comparison to HE RIG animals.

LE RIG group had a greater blood insulin concentration and tended to have a higher glucose concentration. The production and secretion of insulin depend on the rate of nutrient absorption in the small intestine, feed composition, and neuroendocrine signalling to the pancreas (39). LE RIG animals tended to have a higher intake, which could affect appearance of nutrients, including glucose, in the blood. The greater blood glucose would stimulate the secretion of insulin increasing the plasma concentration of this hormone (39).

LE RIG animals had a higher intake with lower ADG. HE RIG animals had a higher BW and heart girth. However, LE RIG group had a greater hip width in comparison to HE RIG animals, even with a lower ADG (Lage et al., 2019). Morphometric differences between RIG groups can indicate differences in body composition between the groups. The deposition of the same weight of lean tissue and fat has different energy costs and studies observed that efficient animals often have greater lean and less fat in the carcass (5,6,40). Greater basal insulin concentration can be related to greater fat deposition since insulin stimulates lipogenesis in adipose tissue (5).

LE RIG had higher left rib, left flank and anus surface temperature measured by infrared thermography in comparison to HE RIG group. In addition, moderate correlations between

flank and rib temperature with RIG were observed. However, no differences in rectal temperature between groups were observed.

This experiment was executed during autumn-winter time and the average THI during analyses was 62.6 ± 4.3 (mean ± SEM), which is within ruminants thermoneutral zone. In this case, lower skin temperatures of more efficient animals should reflect lower heat production for maintenance requirements and less heat being dissipated by radiation (13,37). However, no correlations between thermography and heat production measured by respirometric chamber and no differences in heat production between RIG groups were observed (Lage et al., 2019).

Heat produced by an animal can be associated with several physiological processes, including thermoregulation. Differences in body surface temperature between HE and LE RIG groups can reflect differences in heat loss metabolism. Differences in thermoregulation can be related to energy expenditure and therefore with efficiency. More studies should be done under heat stress condition to better understanding these differences between RIG groups.

Evaluations of thermography during the current study were executed in animals' own stalls. Therefore, animals were subjected to several environmental factors such as, sand, dust, moisture, dirt, and mud. These factors may affect the results.

4.6.FINAL CONSIDERATIONS

The classification of animals in HE and LE groups for FE is feasible since divergence in RFI and RIG were observed in the present trial. Total VFA production and the proportions of acetate and propionate (%VFA) are greater in LE RFI and RFI groups. Therefore, ruminal fermentation does not seem to be responsible for the higher efficiency in pre-weaning heifers. Blood urea concentration was higher in LE RFI animals suggesting that protein metabolism may play a role in efficiency differences. Insulin was greater and glucose tended to be greater for LE RIG animals. Differences in energetic metabolism and body composition may be related

to differences in ADG during pre-weaning phase. Infrared thermography seems to be correlated to RIG but not to HP in pre-weaning calves. Differences in thermoregulation can be responsible for these differences and more studies need to be done for better understanding.

4.7.ETHICS STATEMENT

This study was approved by the Ethics Committee of Embrapa Dairy Cattle (number: 7194210316). The experiment was conducted at the Experimental Farm of Embrapa Dairy Cattle, located in Coronel Pacheco, Minas Gerais, Brazil.

4.8.ACKNOWLEDGEMENTS

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5.0.CONSIDERAÇÕES FINAIS

O uso dos índices de CAR e CGR para classificar bezerras durante a fase de aleitamento resultou em grupos com características diferentes, o que deve ser lado em consideração em pesquisas futuras e programas de seleção animal. A digestibilidade de MS, MO, PB e EE foram os fatores que mais impactaram as diferenças de CAR entre bezerras durante a fase de aleitamento. Apesar da maior ingestão de energia e nitrogênio pelos animais de BE CAR, os animais de AE CAR tiveram a mesma retenção de energia e nitrogênio, o que resultou em mesmo GMD. As concentrações totais de AGV foram maiores nos grupos de BE CAR, o que sugere que os animais que tiveram maior consumo, tiveram maior fermentação ruminal. Portanto, maior fermentação ruminal não parece ser responsável por maior eficiência em animais durante a fase de aleitamento. Ao agrupar os animais baseado no índice CGR, divergência parece estar mais associada às medidas corporais, o que pode estar relacionado a diferenças na composição do ganho. A termografia por infravermelho parece estar correlacionada ao CGR, mas não a PB de calor em bezerros durante a fase de aleitamento e mais estudos precisam ser feitos para melhor entendimento. Pesquisas futuras devem avaliar como o uso dos diferentes índices impactaria em programas de melhoramento genético e quais características relacionadas a eficiencia alimentar quando bezerras estão associadas aos índices de eficiência alimentar dos animais quando vacas.