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EFICIÊNCIA ALIMENTAR E PARÂMETROS PRODUTIVOS EM BEZERRAS F1 HOLANDÊS X GIR

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Eficiência alimentar e parâmetros produtivos em bezerras F1 Holandês x Gir

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RESUMO

Os objetivos foram avaliar se há divergência fenotípica para a eficiência alimentar (EA) na fase de aleitamento, se a EA tem correlação com produção de calor (PC) mensurada pelos métodos de máscara facial (MF) ou termografia infravermelha (TIV) e se estes métodos são aplicáveis em animais em aleitamento. Bezerras F1 Holandês x Gir (n = 36, PN=32,4 ± 6,6 kg) entre a 4ª e 12ª semanas de idade foram classificados em grupos de consumo alimentar residual (CAR) e ganho de peso residual (GPR): alta eficiência (AE, CAR, n = 10 e GPR, n = 9) e baixa eficiência (BE, CAR, n = 10 e GPR, n = 8). A dieta consistiu em leite integral (6 L / d), dieta sólida (95% de concentrado e 5% de feno Tifton 85 picado) e água a vontade. O crescimento e a ingestão de dieta foram monitorados semanal e diariamente, respectivamente. Consumo de O_2 (cO_2), produção de CO_2 (pCO_2) e CH_4 (pCH_4) foram obtidas por MF aos 45 ± 5 dias de idade e a PC foi estimada. As temperaturas máximas foram medidas por TIV aos 62 ± 7 d de idade. Amostras de sangue foram analisadas para glicose, insulina e β-hidroxibutirato (BHBA) e ruminais para pH, ácidos graxos voláteis (AGV), acético, butírico e propiônico, coletadas na 12ª semana de idade. Foi usado delineamento inteiramente casualizado e os dados foram analisados por ANOVA e suas correlações com médias comparadas por teste de Fisher. Houve divergência fenotípica para CAR e GPR. Bezerras AE e BE apresentaram CAR de -0,14 kg/d e 0,13 kg/d e GPR de 0,05 kg/d e -0,07 kg/d, respectivamente. O consumo de matéria seca (CMS) foi 15% menor em AE-CAR. Não houve diferença em CAR no ganho de peso médio diário (GMD), e em GPR para CMS e GMD. Bezerras AE-CAR consumiram menos O_2 (L/d) e produziram menos CO_2 (L/d). A frequência cardíaca (FC) e PC também foram menores. O CAR obteve correlação com PC (r = 0,48), cO_2 (r = 0,48), pCO_2 (r = 0,48) e FC (r = 0,40). Não houve diferença nas trocas gasosas e PC entre os grupos GPR. A temperatura do olho (TO) mensurada pela TIV foi 0,5° C mais alta em AE-GPR. Não houve diferenças em CAR e GPR para pH ruminal, proporção de AGV e proporções molares dos ácidos, assim como glicose, insulina e relação insulina:glicose. Para BHBA e relação glicose:insulina, não houve diferença entre AE e BE para CAR, porém houve tendência a serem maiores em AE-GPR. Houve diferença em CAR para largura inicial do quadril e para GPR na variação da altura de cernelha. Bezerras divergem para CAR, GPR e EA no aleitamento. Os testes de divergência são aplicáveis nesta fase. O método da MF é útil para estimar diferenças na PC entre bezerras com divergência fenotípica para CAR. A TO medida por TIV pode ter potencial para selecionar animais com divergência fenotípica DF para GPR.

Em geral, as estimativas de correlação entre os parâmetros sanguíneos, ruminais e morfométricos foram fracas, e sugerem que é improvável que isoladas, sejam úteis na identificação precoce de animais mais eficientes para índices de EA nesta fase.

Palavras-chave: calorimetria, desempenho, termografia

ABSTRACT

The aims of this study were to assess if there is phenotypical divergence for feed efficiency (FE) during the preweaning phase, if FE is correlated with heat production (HP) measured by the face mask method or by surface skin temperature via thermography, and whether these methods are applicable to preweaned calves it was also evaluated feed efficiency indexes and its effects on body measurements, blood and ruminal metabolites. Holstein x Gyr heifer calves (n = 36, birth BW = 32.4 ± 6.6 kg) enrolled between 4th and 12th w of age were classified into two residual feed intake (RFI) and residual growth (RG) groups: high efficiency (HE; RFI, n = 10; and RG, n = 9), and low efficiency (LE; RFI , n = 10; and RG, n = 8). Calves were fed milk (6 L/d) and solid feed (95% starter and 5% chopped Tifton 85 hay, as-fed). Growth were monitored weekly and feed intake (milk and solid feed) daily, during the whole period. Gas exchanges (O_2 consumption and production of CO_2 and CH_4) were obtained using a face mask at 45 ± 5 d of age and HP was estimated. Maximum temperatures were measured at 7 sites with an infrared camera at 62 ± 7 d of age. Blood samples were collected on 12th w and analyzed for glucose, insulin and BHBA. Rumen samples collected on the same day and analyzed for pH, Total volatile fatty acids (VFA), acetic, butyric, and propionic. A completely randomized design was used, data were analyzed using ANOVA and correlations. Means were compared using Fisher's test. There was divergence in RFI and RG. Respectively, HE and LE calves had RFI of -0.14 kg/d and 0.13 kg/d, and RG of 0.05 kg/d and -0.07 kg/d. Dry matter intake (DMI) was 15% lower in HE-RFI compared with LE-RFI, but there were no differences in average daily weight gain (ADG). Within the RG test, there were no differences in DMI or ADG. HE-RFI calves consumed less O_2 (L/d) and produced less CO_2 (L/d). Heart rate and HP were lower for HE-RFI calves compared with LE-RFI. RFI was correlated with HP (r = 0.48), O_2 consumption (r = 0.48), CO_2 production (r = 0.48), and heart rate (r = 0.40). There were no differences in HP and gas exchanges between RG groups. Methane production was null in both groups. Eye temperature measured by thermography was 0.5°C greater in HE-RG than LE-RG calves. Differences in skin temperature between HE and LE calves were not observed at the other sites. There were no significant differences between the RFI and RG classes for ruminal pH, ruminal NH_3N concentration, proportion of VFA and molar proportions of propionic and acetic acids. For butyric acid no differences were found between RFI groups but tended to be higher in HE residual gain groups. No differences in glucose, insulin and insulin to glucose between the groups were found. For β-hydroxybutyrate

(BHBA) and glucose to insulin no differences exist in RFI groups but tended to be higher in HE residual gain animals. There were also no significant differences between the RFI classes for growth characteristics, except for inicial hip width for RFI group and variation of withers high for RG group ($P < 0.05$). These results support the hypothesis that calves are divergent for RFI, RG and FE during preweaning and divergence tests are applicable during this phase. The face mask method described here is a useful tool for estimating differences in HP among phenotypically divergent RFI calves. Eye temperature measured by IRT may have potential to screen phenotypically divergent RG calves. Overall, the correlation coefficient estimates between the potential blood markers and measurements of rumen and morphometric traits were weak and generally not different from zero. This suggests that it is unlikely that measurement of these metabolic indicators, per se, will be useful in the early identification of feed efficient animals during preweaning phase.

Key words: calorimetry, performance, thermography.

INTRODUÇÃO

A alimentação compreende aproximadamente 60% dos custos do sistema de produção de leite (Ho *et al.*, 2005) e o aumento da eficiência da utilização dos alimentos pode ser uma opção para reduzir esses custos (Berry *et al.*, 2014) e aumentar a eficiência do sistema. Além da menor quantidade de alimento consumido, os animais mais eficientes podem reduzir o impacto ambiental. Uma vez que aproveitam melhor os alimentos, a demanda por uso de terra para pastagens é menor, assim como a emissão de gases de efeito estufa (Waghorn e Hegarty, 2011), que reduziria a contaminação para o meio ambiente.

Por questões ambientais e financeiras, existe crescente interesse em estudos que avaliem características de eficiência alimentar em bovinos de corte e mais recentemente na bovinocultura de leite. Durante os últimos 50 anos os ruminantes foram intensivamente selecionados para uma combinação de características de produção de leite e taxa de crescimento. No entanto, essa seleção não incluiu características de eficiência alimentar, principalmente para bovinos leiteiros, devido aos altos custos para avaliação desses parâmetros (Emmans e Kyriazakis, 2001). Com o avanço de tecnologias de precisão, como cochos eletrônicos, aumentou-se o número de experimentos com eficiência alimentar em bovinos (DeVries *et al.*, 2003; Mendes *et al.*, 2011; Chizzotti *et al.*, 2015; Gregorini *et al.*, 2015) e vários estudos foram realizados em animais em diversas fases do desenvolvimento (Waghorn *et al.*, 2012; Macdonald *et al.*, 2014).

Estudos que avaliaram essas características durante a fase de aleitamento são escassos, por esse motivo devem ser realizados. Evidências indicam que bezerras da raça Holandês selecionadas por divergência em consumo alimentar residual (CAR) durante o crescimento exibem divergências fenotípicas (mesmo que reduzidas) na primeira lactação (Macdonald *et al.*, 2014) e essa variação em CAR tem herdabilidade média (h^2=0,45, Crowley *et al.*, 2010), o que torna possível a seleção de animais com maior eficiência alimentar para compor o rebanho de reposição. A caracterização dessa variável em animais mestiços é importante para que o melhoramento genético com foco em eficiência alimentar seja feito e aumente o potencial econômico dos animais cruzados, uma vez que contribuem com 80% do leite produzido no Brasil. Porém consiste em um contraste, já que a maioria dos experimentos existentes na literatura foram realizadas em animais *Bos taurus*, o que torna essencial a realização desses estudos em animais mestiços.

O mecanismo biológico que controla características de eficiência alimentar não é completamente elucidado, mas inclui diferenças em digestão, demanda fisiológica por nutrientes e eficiência bioquímica da utilização dos alimentos (Herd e Arthur, 2009). De acordo com Paddock (2010), a maior variação de eficiência alimentar entre os animais está provavelmente relacionada a variação nos gastos com energia. O melhor entendimento dos fatores que afetam as diferenças em eficiência alimentar é importante para o desenvolvimento de tecnologias que identifiquem animais mais eficientes precocemente, uma vez que são necessários ensaios experimentais de longo prazo e de alto custo para obter índices de eficiência alimentar (Kelly *et al.*, 2011).

De acordo com Britt *et al.* (2003), animais que têm temperatura corporal mais alta do que outros animais similares sob condições semelhantes, devem utilizar maior quantidade alimentos energéticos na produção de calor metabólico às custas de produtividade. A produção de calor pelos animais pode ser estimada pela dinâmica de troca gasosa, avaliada por câmara respirométrica e/ou máscara (Taylor *et al.*, 1982). Já a termografia por infravermelho é um método utilizado para mensurar temperatura de superfícies, e pode ser uma técnica utilizada para comparar animais mais e menos eficientes, uma vez que, segundo Montanholi *et al.* (2009), animais menos eficientes apresentariam maior temperatura da superfície do corpo do que animais mais eficientes.

Outros fatores estudados para correlacionar com índices de eficiência alimentar são os parâmetros sanguíneos e ruminais, que também podem ser utilizados como ferramentas na identificação de animais mais eficientes, pois podem trazer respostas em relação ao aproveitamento do alimento ingerido. Porém, ainda são escassos os estudos na bovinocultura de leite que relacionem esses parâmetros com CAR e GPR. Embora inúmeros estudos já tenham sido realizados em relação ao CAR em bovinos de corte (Herd e Arthur, 2009; Nkrumah *et al.*, 2006; Montanholi *et al.*, 2010; Basarab *et al.*, 2003), ainda há divergência em relação às diferenças na ingestão e parâmetros ruminais nos diferentes grupos de CAR que ainda necessitam ser definidas mais claramente (Nkrumah *et al.*, 2006; Hegarty *et al.*, 2007).

A fermentação ruminal pode trazer respostas muito interessantes em relação ao CAR, pois a digestão dos alimentos é totalmente dependente da fermentação. Portanto, a busca por resultados dessa relação vem sendo cada vez mais estudadas, a fim de se compreender a maior eficiência dos animais baixo CAR em aproveitar o alimento. Consequentemente, os parâmetros sanguíneos desses animais, já que a avaliação sanguínea colabora na caracterização dos indivíduos, onde a visualização do perfil metabólico ajuda na compreensão

da sua resposta nutricional (Gonzalez *et al.*, 2000). Parâmetros sanguíneos podem auxiliar na identificação de diferenças entre animais na eficiência de utilização de alimentos e ajudar na compreensão de bases fisiológicas ligadas a resposta do metabolismo do animal, alterações metabólicas e perfil hormonal, que contribuiriam assim, para identificação dos animais mais eficientes.

Os objetivos do primeiro artigo deste trabalho foram avaliar se existe divergência para o consumo alimentar residual e ganho de peso residual na fase de aleitamento, e se a produção de calor estimada pelo método da máscara facial e de temperatura superficial pelo método da termografia infravermelha em bezerras F1 podem ser aplicadas na seleção de animais mais eficientes durante a fase de aleitamento e se essas técnicas têm correlação com os índices de eficiência alimentar.

O segundo artigo tem os objetivos de examinar a relação entre a eficiência alimentar e as variáveis metabólicas do sangue e do rúmen, e medidas morfológicas em bezerras classificadas para alta e baixa eficiência para CAR e GPR. Além disso, determinar se as variáveis do sangue e do rúmen podem ser usadas como indicadores de eficiência da utilização de alimentos e se podem ser aplicadas na seleção de bezerras de alta eficiência durante a fase de aleitamento.

CAPÍTULO 1

1. REVISÃO DE LITERATURA

1.1 Medidas de eficiência alimentar

Os índices de eficiência alimentar são calculados de diversas formas, sendo as mais comuns: conversão alimentar (CA) e eficiência alimentar bruta (EAB). Atualmente o consumo alimentar residual (CAR) e o ganho de peso residual (GPR) têm sido estudados para seleção de animais em diversas fases do desenvolvimento. Essas características, em geral, são calculadas pelo consumo de matéria seca (CMS), que representa o *"input"* e características de crescimento, como ganho médio diário (GMD) e peso vivo metabólico ($PV^{0,75}$), representando os *"outputs"* (Grion *et al.*, 2014).

Tabela 1. Índices e respectivas fórmulas para mensuração da eficiência alimentar em animais

Índice	Abreviação	Definição	Fórmula
Eficiência alimentar bruta	EAB	Quantidade de peso vivo ganho com a ingestão de 1,0 kg de alimento (em base seca).	GMD/CMS
Ingestão alimentar	CMS	Consumo alimentar diário (na matéria seca)	
Conversão alimentar	CA	Consumo alimentar por unidade de ganho	CMS / GMD
Consumo alimentar residual (por regressão) (kg/d)	CAR_{reg}	CMS líquida das necessidades esperadas de alimentação para manutenção e crescimento fenotipicamente independente de GMD e PV dos animais.	CMSo (kg) - CMSp (kg), em que a CMSesp é obtido por regressão da CMS no período médio de ensaio com base no $PV^{0,75}$ e GMD
Ganho de peso residual (kg/d)	GPR	Resíduos da regressão de GMD na ingestão de ração e PV metabólico. Trata-se do GMD fenotipicamente indepentente do CMS e PV dos animais.	GMDo (kg/dia) - GMDesp (kg/dia), onde a GMDp é obtido por regressão da GMD no período médio de ensaio com base no valores de CMS e $PV^{0,75}$ de cada indivíduo.
Eficiência parcial de crescimento	EPC	Eficiência do ganho de peso líquido das exigências da energia da mantença (Em)	GMD / (CMS – Em), onde Em obtida por fórmulas (p.ex. NRC, 1996)

CA= conversão alimentar; CAR= consumo alimentar residual; EPC= eficiência parcial de crescimento; EFM= eficiência para mantença; GR= ganho residual; CGR= consumo e ganho residual;

CMS= consumo de matéria seca; CMSo= CMS observado; CMSp= consumo de matéria seca predito; $PV^{0,75}$= peso vivo metabólico médio; GMD= ganho de peso diário; GPDo= ganho de peso diário observado; GPDp= ganho de peso diário predito; CMSm= consumo de matéria seca para mantença; PV= peso vivo; Adaptado de Herd e Arthur, 2009.

Eficiência alimentar não pode ser diretamente mensurada, mas pode ser calculada em função do tempo, alimento consumido e ganho de peso (Koch *et al.* 1963). Eficiência alimentar tem maior correlação com CAR quando comparado a eficiência de conversão alimentar (Arthur *et al.*, 2001a). A variação na EA pode ser resultado dos diferentes ambientes em que os animais foram avaliados (Koch *et al.* 1963). A seleção para conversão alimentar (CA) pode resultar em maior peso adulto (Crews, 2005). Arthur *et al.* (2001a) encontraram herdabilidade para CA de 0,29 (Arthur *et al.*, 2001 a). Como a seleção para menor CA pode resultar em animais maduros mais pesados, com maiores taxas de crescimento e requisitos de mantença maiores (Crews, 2005), aumentar o peso final do animal adulto não irá resultar em um animal que utiliza o alimento da maneira mais eficaz, já que terá como resultado o aumento das exigências de mantença. De acordo com Mader *et al.* (2009), há correlação negativa entre GMD e deposição de gordura na carcaça, o que sugere que a quantidade interna de deposição de gordura em novilhas de corte tem impacto negativo no GMD. As mudanças na deposição de gordura e músculo influenciam a eficiência durante o crescimento, uma vez que a deposição muscular requer menos energia da dieta do que a deposição de gordura (Mader *et al.*, 2009).

A eficiência alimentar bruta é calculada pela razão entre o ganho de peso e o consumo de matéria seca e, portanto, animais com EAB superior são considerados mais eficientes. Por ser uma razão direta entre ganho de peso e consumo de matéria seca, estas características, podem selecionar animais mais pesados, que não necessariamente apresentam menor consumo de matéria seca (Arthur *et al.*, 2001 a). A utilização destas medidas compromete a eficiência produtiva de sistemas de produção, por haver aumento no tamanho adulto dos animais e, consequentemente, das suas exigências de mantença, além de comprometer a eficiência reprodutiva em condições nutricionais limitantes.

Indivíduos com mesmo peso vivo consomem quantidades muito diferentes de alimento para o mesmo patamar de produção. Como fortalecimento dessa ideia, Koch *et al.* (1963) reconheceram que as diferenças nos dois principais requisitos de mantença, peso e ganho de peso, afetavam o consumo. Os autores sugeriram que o consumo deveria ser ajustado para peso vivo e ganho de peso e efetivamente dividido em dois componentes: 1) consumo

previsto para o patamar de produção (ganho de peso e peso) e mantença, e 2) uma parcela residual, que poderia ser utilizada para identificar animais que tenham desvio do patamar de consumo esperado, e denominaram esta nova característica de consumo alimentar residual (CAR). Em sua definição é a diferença entre o consumo real e o consumo predito, por equação de regressão linear do consumo individual real em função do peso vivo metabólico médio ($PV0^{,75}$) e ganho de peso médio diário (GMD). O resíduo dessa equação é o valor do CAR, que por sua vez, pode ser positivo ou negativo.

Portanto, com a característica CAR, é possível identificar animais que apresentem diferença entre a exigência nutricional estimada e observada e classificá-los. Animais eficientes apresentam baixo CAR e tem consumo observado menor que o estimado, ao contrário dos menos eficientes que tem alto CAR e consumo observado maior que o estimado. Em geral, estudos envolvendo a caracterização de CAR classificam os animais em grupos de eficiência baixo, médio e alto CAR (Del Claro, 2011).

O CAR tem herdabilidade moderada (h^2= 0,29-0,46) em bovinos de corte (Arthur et al, 2001a; Arthur et al, 2001b) e variação genética suficiente para respostas favoráveis a ocorrência de seleção genética (Herd e Bishop, 2000). Estima-se que a herdabilidade para CAR está entre 0,13 e 0,82 (Berry e Crowley, 2013a,b). Nkrumah *et al.* (2007) relataram estimativa de herdabilidade para CAR de 0,42 em machos castrados provenientes de cruzamentos entre *Bos taurus*. Trabalhos que relatam estimativas de herdabilidades em animais *Bos indicus* são escassos. Contudo, Santana *et al.* (2014), em experimento com machos da raça Nelore, estimaram herdabilidade de 0,37 para CAR.

Vários estudos que aplicam o conceito de CAR têm sido conduzidos para elucidar algumas das diferenças metabólicas que poderiam ser potenciais preditores de eficiência alimentar, que vão desde estudos sobre a função de organelas celulares ao metabolismo energético (Swanson e Miller, 2008). Estudos sobre o metabolismo da energia têm demonstrado que os animais mais eficientes têm menor perda de calor e menor produção de metano (Nkrumah et al, 2006). No entanto, a ferramenta CAR não tem sido amplamente implantada como para seleção devido ao alto custo, relativo a mensuração de consumo individual dos animais por determinado período, fato que limita o uso dessa medida por sistemas de produção comerciais (Paddock, 2010).

Com princípio semelhante ao utilizado na definição do CAR, Koch *et al.* (1963) também propuseram o ganho de peso ajustado para o consumo, conhecido como ganho de peso residual (GPR). O GPD é calculado como o resíduo da regressão linear do GMD em função

do $PV^{0,75}$ e do consumo de matéria seca, ou seja, o índice avalia o ganho de peso para cada kg de ingestão de alimento, considerando o mesmo peso corporal (Crowley *et al.*, 2010). Neste caso, os fenótipos desejados são os animais com GPR positivos, ou seja, aqueles que ganham mais peso que o esperado para o $PV^{0,75}$ e o CMS observados e, portanto, são mais eficientes que seus contemporâneos (Berry e Crowley, 2013a,b).

Crowley *et al.* (2010), consideraram o GPR altamente correlacionado com o ganho de peso e mencionaram que o índice pode ser confundido com as características produtivas. Os autores associaram GPR com as taxas de crescimento mais rápidas, mas não obtiveram diferença no consumo de alimento entre os animais avaliados. O GPR é expresso em kg de ganho por dia e valores elevados são desejáveis (Grion, 2012). O GPR permite identificar animais eficientes com elevada taxa de crescimento e não apresenta correlação com peso corporal (Berry e Crowley, 2013a,b).

Avaliar esse índice e caracterizar o ganho de peso para cada kg de consumo de alimento pode ser uma métrica interessante, já que os crescentes custos de alimentação são fatores direcionadores para a busca da eficiência nos sistemas de produção. Existem poucas estimativas de herdabilidade para a característica GPR na literatura, Koch *et al.* (1963) encontraram herdabilidade de 0,62 e Crowley *et al.* (2010) de 0,28 para animais taurinos e para zebuínos, enquanto Santana *et al.* (2014) estimaram herdabilidade de 0,40.

Crowley *et al.* (2010) relataram correlação genética de -0,03 entre GPR e CMS, e indicou que essa característica de eficiência alimentar é independente geneticamente do consumo dos animais e não é útil para identificar diferenças no consumo de alimentos entre os indivíduos, que também é uma variável determinante de lucro. Porém, os autores encontraram diferença na média ($P<0,05$) de CMS de animais alto GPR e baixo GPR. De acordo com Crowley *et al.* (2010), entre as definições de eficiência alimentar ainda é incerta a variável de estudo de eficiência alimentar que melhor se adequa aos sistemas de produção de leite, e é necessário melhor entendimento dos fatores que regulam a eficiência alimentar e seu potencial como preditores de bovinos mais eficientes (Montanholi *et al.*, 2010).

1.2 Fontes de variação biológica em consumo alimentar residual (CAR)

De acordo com Herd *et al.* (2004), em estudo sobre os fatores biológicos que contribuem para a variação observada no CAR em gado de corte podem advir da digestão (14%), do incremento calórico (9%), composição corporal (5%) e diferenças de atividade física (5%).

Desta forma, 67% da variação restante em CAR em bovinos de corte permanece inexplicável e pode estar relacionada com a energia requerida pelos processos biológicos, como bombeamento de prótons na mitocôndria, *turnover* proteico e bombeamento de íons. Brosh (2007) propôs que os principais fatores que afetam a partição de energia nos ruminantes são: patamar de consumo alimentar, condições ambientais, gasto energético ou produção de calor, patamar de produção de leite ou ganho em tecido corporal e variabilidade individual entre animais quanto a eficiência de utilização de energia para mantença e produção.

De acordo com Nkrumah *et al.* (2006), animais com baixo CAR tiveram menores perdas de energia fecal e metano, mas perdas energéticas via urina semelhante aos animais alto CAR, o que correspondeu a diferença de 6,3% na energia metabolizável entre animais baixo e alto CAR. Hegarty *et al.* (2007) também encontraram diferença na perda de energia em emissão de metano em animais alimentados a vontade. Paddock (2010), após contabilizar estas diferenças concluiu que a maior variação do CAR é provavelmente relacionada com a variação dos gastos de energia. Castro Bulle *et al.* (2007), ao calcularem o equilíbrio energético do animal, a produção de calor representou um componente substancial do balanço de energia dos ruminantes (). Esses autores afirmaram ainda que animais de baixo CAR utilizaram menos energia nos processos fisiológicos que envolvem a mantença, o que resultou em maior energia líquida para acréscimo de tecido, ou seja, o CAR pode ser correlacionado negativamente com o requerimento de energia de mantença.

Por definição, a soma dos gastos com energia associada ao metabolismo basal, respostas termorreguladoras e atividades físicas, além de energia retida como o produto (por exemplo, leite, tecido) será igual a ingestão total de energia metabolizável por um animal. Estudos com bovinos de corte em crescimento mostraram que 4 a 9% das variações no CAR estão associadas a diferenças na composição da carcaça (Basarab *et al.*, 2003; Lancaster *et al.*, 2009).

Os mecanismos fisiológicos identificados até então, são baseados em poucos estudos, e desses, alguns com baixo n amostral. Os maiores desafios são desvendar os mecanismos responsáveis pela variação que continua inexplicável, validar proporcionalmente as contribuições e estabelecer relação entre as informações fisiológicas com as de genética molecular propostas atualmente (Herd e Arthur, 2009).

1.3 Produção de calor

A produção de calor 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). De acordo com Brosh e Aharoni (2005), a mensuração do gasto energético pode permitir a determinação da eficiência individual, na qual é conferida maior eficiência de utilização da energia ao animal com o menor gasto energético em relação a sua produção. 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. Após a ingestão de alimentos ocorre o gasto energético extra, denominado incremento calórico, que inclui calor gerado na digestão, absorção e fermentação (Cannas *et al.*, 2010). Segundo o NRC (2000), o incremento calórico é calculado pela diferença entre a produção de calor do animal alimentado e em jejum ($IC = PC_{alimentado} - PC_{jejum}$). A variação na perda de energia proveniente do incremento calórico depende da quantidade e da qualidade da dieta fornecida. O aumento do incremento calórico está diretamente relacionado ao aumento do consumo ou a melhoria da composição química da dieta, visto que os processos de digestão e transporte da digesta no trato digestivo requerem energia (Chwalibog, 2004).

O custo energético de mantença, perdido na forma de calor, pode representar até 75% do consumo total de energia (Ferrell e Jenkins, 1984; NRC, 1984). Com isso, um esforço significativo vem sendo empregado na tentativa de desenvolver métodos que permitam estimar a produção de calor 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).

1.4 Técnicas para mensurar a produção de calor

Em estudos de metabolismo energético, a produção de calor dos animais é estimada como produção de calor total, que inclui o calor utilizado para mantença, adicionado ao calor despendido na forma de incremento calórico (Rodriguez *et al.*, 2007). A exigência de mantença pode ser definida como a quantidade de energia necessária para animais com ganho de peso nulo (Ferrell e Jenkins, 1984). Essas necessidades representam a quantidade de energia necessária para manter os processos do metabolismo basal (síntese e degradação proteica, transporte de íons, sinalização celular), função de órgãos vitais, movimentos voluntários e termorregulação (Thompson *et al.*, 1983).

O custo energético de mantença e as diferenças nas exigências de mantença (10 a 12 % em bovinos de corte) são componentes chaves que definem as variações na eficiência entre animais (Swanson e Miller, 2008). Estas variações, em conjunto com as estimativas de herdabilidade sugerem amplo espaço para melhorias através de seleção de animais com menor exigência de mantença, ou seja, animais que perdem menos energia na forma de calor (Bishop *et al.*, 1991).

As técnicas mais utilizadas para quantificar a produção de calor em ruminantes se baseiam no abate comparativo de animais ou uso de câmaras respirométricas. Em abates comparativos, os animais ou amostra representativa são abatidos e a composição do corpo vazio é determinada. A composição inicial dos animais remanescentes é estimada por equações de regressão, derivadas a partir dos resultados obtidos de abate do grupo referência que relacionam o peso da carcaça ao conteúdo corporal de energia, proteína e gordura, com o peso vivo em jejum (Corbertt e Ball, 2002). Assim, é possível calcular indiretamente a produção de calor, já que o consumo de energia metabolizável (obtido em ensaio de metabolismo) é igual a produção de calor mais a energia retida. O abate comparativo permite a determinação da produção de calor em condições de produção, no entanto, requer o sacrifício dos animais, fator limitante para a realização de experimentos com vacas lactantes.

A avaliação da produção de calor via calorimetria indireta em câmara respirométrica baseia-se na mensuração das concentrações dos gases O_2, CO_2 e CH_4 no ar inspirado (que entra na câmara) e expirado (que sai da câmara), e não exige abate dos animais. A Equação de Brouwer (1965) é utilizada para calcular a produção de calor a partir do consumo de O_2, produção de CO_2 e CH_4, e da concentração de N urinário. A calorimetria indireta é uma

técnica que quantifica o calor gerado pelas reações químicas, ou seja, é uma medida metabólica da oxidação de compostos orgânicos, mas que permite avaliação de número limitado de animais. Foi desenvolvida depois do século XIX como uma aplicação da termodinâmica para vida animal (Van Soest, 1982). É baseada no conhecimento da combustão do substrato energético ingerido e necessário pelo organismo.

Os diferentes tipos de nutrientes têm quantidades específicas de O_2 consumido e CO_2 produzido, portanto, a produção de calor é obtida pelo quociente respiratório (QR), o qual é determinado pelo balanço conjunto de oxigênio e carbono, ou das perdas e ganhos de tecidos pelos animais (Van Soest, 1982). Existem diversas formas de se medir as trocas gasosas na respiração.Todas levam em consideração o consumo de oxigênio (VO_2) e a produção de CO_2 por unidade de tempo.

O método pulso de oxigênio e frequência cardíaca (O_2P-HR) é uma técnica alternativa que permite medir o gasto energético de bovinos em condições mais próximas do ambiente em que são criados (Brosh, 2007). É baseado na mensuração da frequência cardíaca (em três períodos de 24 horas) e consumo de O_2 (20 minutos) obtido pelo método da máscara facial. Oss *et al.* (2016) demonstraram que o O_2P-HR, com uso da máscara facial para mensurar o VO_2, proporcionou estimativas de produção de calor similares aos valores obtidos em câmaras respirométricas e pela metodologia do abate comparativo para touros leiteiros jovens. Os métodos tradicionais que utilizam a calorimetria indireta para mensurar o gasto energético são as câmaras calorimétricas de circuito aberto onde os animais são alojados individualmente.

Embora seja uma medida acurada, essas câmaras não representam o real ambiente do animal e restringem as atividades dos animais, o que pode afetar as exigências de energia e seu uso de acordo com NRC (1996). Embora a produção de calor de um animal possa ser medida de forma acurada por meio de câmara calorimétrica, estes métodos, além de serem praticados em condições artificiais, são extremamente caros e requerem considerável experiência e infraestrutura, o que pode tornar impraticável seu uso para seleção de animais com menor exigência.

1.5 Relação entre produção de calor e índices de eficiência alimentar

Nkrumah *et al.* (2006) relataram que a produção de calor foi 21% menor para animais baixo CAR em relação ao alto CAR, e 10% menor para animais médio CAR comparados ao alto

CAR. Os autores mostraram que um dos fatores que pode explicar a melhor eficiência dos animais baixo CAR é o menor gasto energético com produção de calor.

Hafla *et al.* (2013) não estudaram a relação do CAR com a produção de calor, mas observaram como a classificação de CAR pode afetar algumas características de desempenho em 48 novilhas Bonsmara durante a gestação, como a frequência cardíaca (FC). Os autores observaram que a FC das fêmeas classificadas em baixo CAR foi 7 % menor em relação às fêmeas em alto CAR (66,1 vs 71,1 bat/min).

Paddock (2010) estudou 16 novilhas Brangus selecionadas para alto e baixo CAR e observou maior FC nos animais de alto CAR (97,7 vs 89,6 bat/min). Além disto, as novilhas com alto CAR consumiram mais oxigênio por batimento cardíaco (ml/bat), como resultado, o gasto energético (produção de calor) foi 17,4 % maior nos animais de alto CAR do que nos de baixo CAR.

Basarab *et al.* (2003) avaliaram 176 novilhos mestiços e concluíram que os mais eficientes (baixo CAR) apresentaram menor consumo de energia metabolizável (10,2%), menor retenção de energia (12%), produziram menos calor (9,3%) e apresentaram vísceras menores (fígado, abomaso e intestinos) do que os menos eficientes (alto CAR).

De acordo com VandeHaar *et al.* (2016), vacas com baixo CAR provavelmente digerem e metabolizam nutrientes de forma mais eficiente e têm maior eficiência e rentabilidade global se também são saudáveis e férteis. Assim, espera-se menor incremento calórico em animais que comem menos pelo mesmo desempenho (Herd e Arthur, 2009).

De acordo com Nkrumah *et al.* (2006), a identificação das razões metabólicas e fisiológicas que afetam a variação na EA em bovinos que são semelhantes em PV e crescimento é um pré-requisito, que deve ser bem reconhecido para o planejamento efetivo de estratégias de melhoramento para selecionar animais que sejam mais eficientes. Os autores consideraram várias vias metabólicas e fisiológicas que potencialmente poderiam influenciar a EA. Essas vias foram correlacionadas às variações na eficiência da conversão de energia bruta em energia metabolizável (devido às diferenças na digestibilidade, geração de gases durante a fermentação ruminal, absorção de nutrientes, excreção de resíduos e produção de calor) e a subsequente eficiência da conversão de energia metabolizável em energia retida para manutenção e crescimento.

1.6 Termografia infravermelha

A temperatura termodinâmica pode ser caracterizada como um indicador da quantidade de energia contida em um corpo e é habitualmente medida em escala relativa de graus Celsius (°C) ou graus Fahrenheit (°F) (Stelletta *et al.*, 2012). Abordagens para aplicação de métodos de fácil execução a fim de prever indiretamente a eficiência dos animais têm sido estudadas. Termorregulação é um fator relacionado a eficiência alimentar, pois está associada ao metabolismo energético e a produção de calor (Herd *et al.*, 2004). Assim, a termografia utilizada para demonstrar a termorregulação animal poderia ser explorada para esta finalidade.

A TIV é a técnica de inspeção não invasiva, realizada por intermédio de sistema infravermelho (IV) para a mensuração de temperaturas ou observação de padrões diferenciais de distribuição de calor Seu objetivo é fornecer informações relativas às condições térmicas de superfícies. O IV é uma frequência eletromagnética naturalmente emitida por qualquer corpo, com intensidade proporcional a sua temperatura. Assim, o termovisor, facilita a localização de regiões quentes ou frias pela interpretação dos termogramas que fornecem imagens, em faixas de temperatura que podem cobrir de - 40 a 1500ºC (Colyn, 2013).

As variações de temperatura da pele podem refletir estado de integridade ou injúria nos tecidos, provenientes de condições circulatórias e metabólicas. Com isso, torna-se possível inferir sobre condições fisiológicas e alterações vasculares locais (Stelletta *et al.*, 2012).

Temperaturas das porções distais da garupa e cabeça foram os locais mais adequados do corpo para avaliar indiretamente a eficiência alimentar em bovinos através de termografia (Montanholi *et al.*, 2009). Segundo os autores, as extremidades corporais são as regiões mais indicadas para avaliação pois são onde acontecem as maiores trocas de temperatura com o meio.

A TIV é alternativa aos métodos invasivos comumente usados para avaliação fisiológica e de parâmetros metabólicos. Esses por sua vez, podem ser alterados por respostas ansiogênicas resultantes dos procedimentos para sua obtenção (Soerensen e Pedersen, 2015).

A avaliação da temperatura superfícial do animal pode também ser utilizada como indicador para estimar o estado fisiológico dos animais em situações de estresse, avaliação complementar da fertilidade, do bem-estar e da saúde animal (McManus *et al.*, 2015). Baseada na mensuração do perfil térmico de uma superfície e no fato de que todos os corpos

geram calor e emitem radiação IV, a TIV pode ser realizada e correlacionada com a temperatura corporal (Knizkova *et al.*, 2007).

1.7 Relação entre termografia infravermelha e índices de eficiência alimentar

Previamente Montanholi *et al.* (2007) sugeriram que animais mais eficientes apresentavam menor temperatura de superfície corporal que animais menos eficientes (alto CAR). Montanholi *et al.* (2008) propuseram a utilização da TIV, em diferentes pontos corporais, para predizer a produção de calor e detecção de eventos fisiológicos como incremento calórico na alimentação em vacas em lactação. Os autores utilizaram quatro vacas e imagens termográficas foram tiradas dos flancos esquerdo e direito, garupa e membros anteriores (face traseira). Correlações entre 0,58-0,88 foram observadas entre as temperaturas da superfície da pele e a produção de calor. As temperaturas observadas nos membros anteriores tiveram alta correlação com a produção de calor (esquerda: 0,83; direita: 0,88; $P < 0,001$). Flanco direito e garupa tiveram correlações mais baixas com a produção de calor quando comparadas às dos membros anteriores. Os autores concluíram que os membros poderiam ser o local mais adequado para previsão de produção de calor pelo método da TIV.

O fato de que a garupa e flanco esquerdo mostraram correlações mais baixas com produção de calor do que os outros locais do corpo não foi surpreendente para os autores, já que previamente, Montanholi *et al.* (2007) não encontraram diferenças na temperatura deste local do corpo em novilhos divergentes para CAR, mas sim na temperatura de globo ocular. Em estudo anterior (Montanholi *et al.*, 2006), a temperatura da superfície da garupa obteve maior repetibilidade em medidas de dois dias consecutivos, e sugeriu que este local tem uma temperatura relativamente estável e pode não refletir flutuações na produção de calor.

Porém Schaefer *et al.* (2005), tentaram correlacionar imagens termográficas com a classificação divergente para CAR em vacas e avaliaram a temperatura do dorso dos animais em três datas em um teste com duração de 84 dias. Segundo a classificação de baixo, médio e alto CAR, a temperatura máxima da superfície dorsal foi significativamente menor nas vacas baixo CAR em relação aos animais alto CAR. Isto sugere que a utilização de análises de TIV pode apresentar utilidade na avaliação da eficiência de vacas em lactação. Montanholi *et al.* (2009) relataram que a extremidade dos membros posteriores e a temperatura da mandíbula são os locais do corpo mais indicados para avaliar indiretamente a EA em bovinos.

Ao avaliar o melhor tempo ao longo do ciclo circadiano e o número adequado de termogramas ao longo do tempo, Montanholi *et al.* (2009) atestaram a possibilidade de aumento da previsibilidade de EA com uso da TIV O que pode resultar na aplicação desta tecnologia na seleção de bovinos mais eficientes. Essa avaliação indireta da EA seria mais barata e com menor limitação de predição quando comparada a medida de consumo de alimento.

Huntington *et al.* (2012) avaliaram o CMS, GMD e EA em touros Angus (n = 277, 60-81 touros por ano), durante 4 anos. Imagens termográficas da área da costela esquerda foram registradas para cada touro, CAR variou entre -2,17 a 3,07 kg/d (desvio padrão = 0,55). CAR foi positivamente correlacionado ($P < 0,05$) com CMS (0,49) e negativamente correlacionado com EA (-0,50), mas não foi correlacionado com as medidas termográficas. CMS diária teve correlação negativa ($P < 0,05$) com termografia ($r=-0,15$ a $-0,55$). Entre as áreas de costela, temperaturas de mínima, máxima e as médias foram positivamente correlacionados umas com as outras ($0,34 \leq r \leq 0,93$) e com o GMD ($P < 0,05$). De acordo com os autores, as correlações significativas entre temperaturas de superfície e CMS, GMD e EA indicaram que a TIV poderia ser utilizada para avaliar o estado ou o progresso da taxa e eficiência de ganho individuais de animais confinados.

Colyn (2013) determinou CAR de 61 novilhas de corte em ensaio de 113 dias após o desaleitamento. Os valores de CAR variaram de -1,55 a 2,19 kg/d (desvio-padrão = 0,78). Novilhas foram classificadas em baixo, médio ou alto CAR. Média das temperaturas das imagens termográficas de diversos pontos foram feitas de quatro momentos. Os animais baixo e médio CAR tiveram menor CMS: 7,5 e 6,5%, respectivamente, comparados a animais alto CAR. Novilhas de baixo e médio CAR obtiveram temperaturas média de ganacha semelhantes de 19,88ºC e 20,40ºC, porém menores do que novilhas alto CAR (21,29ºC; $P <0,0001$). Média das temperaturas de globo ocular tenderam a crescer nos grupos (de baixo a alto) $P= 0,0747$. Houve correlação de $r = 0,46$ ($P < 0,001$) da temperatura da mandíbula com CAR. Dessa forma, o autor concluiu que a mensuração de perda de calor irradiado pelo método da TIV na área da ganacha poderia predizer a eficiência em novilhas. Contudo, sugerem que mais ensaios devam ser realizados para garantir a correlação. Em outro estudo (Montanholi *et al.*,2010) as temperaturas de ganacha encontradas foram correlacionados positivamente com o CAR, CMS, GMD, CA em bovinos de corte.

Montanholi *et al.* (2010) avaliaram potenciais preditores de EA pela TIV da área do globo ocular, ganacha, focinho e costelas, em 91 novilhas durante dois períodos de ensaio (ano 1=

46; ano 2=45) com duração de 140 dias. Novilhas foram classificadas como baixo, médio e alto CAR. Os animais baixo CAR tiveram CMS e temperaturas de ganacha e focinho menores que animais menos eficientes (alto CAR) (28,1º C x 29,2º C e 30,0º C x 31,2º C, respectivamente), que indicam melhor eficiência energética nos animais baixo CAR.

Martello *et al.* (2015) avaliaram o uso da TIV como ferramenta de monitoramento da temperatura de superfície corporal e correlacionaram com CAR na raça Nelore (n = 144). Com objetivo de estudar também a termografia como indicador de EAem *Bos indicus*, animais classificados como alto CAR e baixo CAR foram avaliados. Foram feitas: mensuração de temperatura retal, frequência respiratória, e imagens termográficas da cabeça (fronte), olho, zona ocular, ganacha, flanco, costelas, garupa e patas dianteiras. As temperaturas termográficas medidas no olho, cabeça (fronte), flanco, costelas, garupa, e os pés foram positivamente associados com frequência respiratória e temperatura retal. Os achados indicaram que o aumento de temperatura nas imagens termográficas está associado com o aumento da frequência respiratória e temperatura retal. Houve efeito no grupo CAR na TIV da região da fronte, que se correlacionou positivamente com temperatura retal. A TIV da fronte para animais alto CAR foi menor ($P < 0,01$) que para animais baixo CAR. A temperatura da pele mais elevada mensurada por termografia para os animais do grupo baixo CAR pode ser relacionada a melhor eficácia dos mecanismos de termorregulação, pois a temperatura retal permaneceu menor no grupo de baixo CAR. Os autores concluíram que a TIV da cabeça (fronte) pode ser usada para estudos relacionados com CAR em gado de corte.

Contudo, mais estudos devem ser realizados para correlacionar TIV e CAR, a fim de se obter melhor compreensão da dinâmica de termorregulação, principalmente emno que diz respeito ao estudo de animais *Bos indicus* em países de clima tropical.

1.8 Relação entre parâmetros ruminais e índices de eficiência alimentar

A avaliação dos parâmetros ruminais é de grande importância em animais classificados para diferentes índices de EA, pois esses p podem indicar a eficiência na utilização do alimento através da digestibilidade (Baldwin e Allison, 1983). A digestão dos nutrientes por ruminantes é dependente da fermentação ruminal e pode haver diferenças nas variáveis de fermentação entre animais de alta e baixa eficiência para CAR. A população microbiana do rúmen pode afetar o pH, os ácidos graxos voláteis (AGV, o nitrogênio amoniacal (N-NH3) e alterar a taxa de digestão da dieta. Estabelecer a relação entre o CAR e fermentação ruminal é de grande

relevância, principalmente para melhorar o conhecimento de parâmetros biológicos que contribuem para a variação do CAR (Trevizan, 2016).

Richardson e Herd (2004) e Herd e Arthur (2009) sugeriram que a maior eficiência dos animais classificados como baixo CAR está associada com a maior capacidade de digestão da dieta. Segundo Richardson *et al.* (2004), variações individuais observadas na digestibilidade dos alimentos estão associadas a taxa de passagem destes pelo trato-gastrointestinal. Animais alto CAR apresentaram maior taxa de passagem e, portanto, menor digestibilidade dos alimentos. Os autores supracitados observaram correlação negativa e significativa do CAR com a taxa de passagem dos alimentos de 0,44.

De acordo com Khan *et al.* (2016), o desenvolvimento do rúmen ocorre com a presença e estabelecimento do ecossistema microbiano, muscularização e vascularização da parede ruminal, desenvolvimento papilar e o início da ruminação e da motilidade do rúmen. Não impulsionado portanto somente pela presença de ácidos graxos voláteis (AGV), mas também pelos nutrientes fornecidos a partir da dieta líquida, de forma que o desenvolvimento do rúmen e do intestino delgado são vinculados.

Richardson *et al.* (1996) avaliaram machos e fêmeas jovens classificados em alta e baixa eficiência para CAR. Apresentaram tendência em sua capacidade de digerir matéria seca e esta diferença na digestibilidade foi responsável por 14% da diferença de consumo entre alto a baixo CAR. A digestão de nutrientes pelos ruminantes é em grande parte dependente de fermentação ruminal, que consequentemente melhora a digestão da dieta e conduz a melhoria da EA.

De acordo com Teixeira e Teixeira (2001) a população microbiana ruminal converte os carboidratos fermentados em 60 a 70% de ácido acético, 18 a 22% de ácido propiônico e 13 a 16% de ácido butírico. Eem dietas de maior teor de concentrado essa relação pode chegar a 40:40:20 (Bergman *et al.*, 1990). Ao estudar as concentrações de amônia e pH ruminal, pode-se compreender melhor a eficiência na utilização dos alimentos, devido ao fornecimento de informações do processo fermentativo (Nagaraja *et al.*, 1997).

A quantidade de nitrogênio amoniacal (N-NH3) no rúmen é um fator importante, pois os microrganismos ruminais utilizam como fonte de N para síntese proteica (Oliveira *et al.*, 2013). Para que a produção de proteína microbiana no rúmen seja otimizada é necessário que haja equilíbrio entre a quantidade de N e de energia disponíveis no rúmen (Firkins, 1996). De acordo com Van Soest (1982), a concentração ótima de amônia para fermentação ruminal é de 10 mg/ 100 mL. As bactérias ruminais podem utilizar os aminoácidos tanto para síntese de

proteína microbiana quanto fermentá-los para utilizá-los como fonte de energia (Ribeiro *et al.*, 2001).

Segundo Mathis *et al.* (2000), a presença dos compostos nitrogenados dos substratos da dieta está correlacionada com a digestibilidade dos alimentos. As bactérias, fungos e protozoários que habitam o ecossistema ruminal diferem nas necessidades de nutrientes e metabolismo (Bach *et al.*, 2005). Esses microrganismos tem a capacidade de sintetizar todos os aminoácidos essenciais (Nolan e Dobos, 2005) e a síntese desses aminoácidos é efetuada a partir da amônia e esqueletos de carbono produzidos durante a degradação do alimento. A possibilidade de utilizar a amônia permite aos microrganismos do rúmen reciclar quantidades importantes de ureia, provenientes do metabolismo intermediário como fonte de nitrogênio para a síntese de proteína microbiana. Desta forma, deve-se buscar encontrar a relação do CAR com os parâmetros de fermentação ruminal e metabolismo animal para obter respostas em relação a eficiência alimentar dos animais (Trevizan, 2016).

1.9 Relação entre parâmetros sanguíneos e índices de eficiência alimentar

De acordo com Bellmann *et al.* (2004), hormônios são componentes influenciados diretamente por fatores nutricionais, pois regulam a partição de nutrientes (insulina e glucagon) e determinam a taxa de metabolismo basal ou de deposição (hormônios do eixo somatotrópico, hormônio de crescimento, IGF-1 e hormônios da tireóide).

A insulina influencia a regulação da concentração de glicose circulante e é diretamente envolvida com o crescimento celular e desenvolvimento dos animais (Fouladi-Nashta e Campbell, 2006). Atua também no hipotálamo, sobre o mecanismo da fome-saciedade e em tecidos corporais como fígado, músculos, glândulas mamárias e ovários (Cunningham, 2004; Volp *et al.*, 2008).

O IGF-1 é liberado pelo fígado e tecidos periféricos. Atua na concentração de glicose e metabolismo de aminoácidos e proteínas, com alteração nos processos de síntese e degradação, influencia no crescimento, composição de carcaça e EA (Lobley, 1992).

Kelly *et al.* (2010) analisaram o plasma sanguíneo de novilhas cruzadas Limousin x Holandês e encontraram correlações significativas entre CAR e as concentrações de ácidos graxos não esterificados (AGNE) (r = -0,21) e β-hidroxibutirato (r = 0,37). Além de correlações significativas entre CA e as concentrações sanguíneas de leptina (r = 0,48), AGNE (r = -0,32), β-hidroxibutirato (r = 0,25) e relação glicose: insulina (r = -0,23), e concluíram que os

processos biológicos do animal podem ser responsáveis pela variação da EA em gado de corte.

Santos (2014), com o objetivo de avaliar associações entre desempenho, EA, parâmetros sanguíneos e CAR em novilhos Nelore, classificou os animais em baixo (CAR < -0,128 kg/d; n= 40); médio (CAR entre -0,128 e 0,135 kg/d; n= 42) e alto CAR (CAR > 0,135 kg/d; n= 36). Animais baixo CAR consumiram, em média, 0,670 kg/d de matéria seca a menos que animais alto CAR. Dos parâmetros sanguíneos analisados, a ureia, IGF-I e insulina apresentaram diferenças entre os grupos. Foram detectadas concentrações sanguíneas menores de ureia (5,58 vs 5,91 mmol/L) e maiores de insulina (4,45 vs 3,70 µIU/mL) e IGF-I (433 vs 399 ng/mL), para animais baixo CAR quando comparados aos animais alto CAR, respectivamente.

Richardson e Herd (2004) encontraram que animais mais eficientes tiveram menores níveis sanguíneos de ureia, cortisol e insulina, e maiores níveis de triglicerídeos, estas respostas possivelmente estão relacionadas com a reciclagem dos tecidos, em mudanças na composição corporal e a uma resposta ao estresse.

De acordo com esses autores, as diferenças mitocondriais entre os animais podem ser uma explicação para a diferença de eficiência existente. Diferenças mitocondriais se relacionam com as medidas fenotípicas de eficiência. Foi encontrada relação entre o VO_2 e o CAR do animal. Kerley (2010) observou que animais mais eficientes tiveram VO_2 mais rápido e conseguiram estabelecer a homeostase da fosforilação mais rapidamente que animais menos eficientes, além de cessaram o consumo em menos tempo, por alcançarem saciedade. Isso explicaria o consumo reduzido, a existência de menor gordura corporal e glicemia e concentrações de insulina diminuídas em animais baixo CAR.

1.10 Relação entre características morfológicas e índices de eficiência alimentar

Características morfológicas estão relacionadas a longevidade, funcionalidade e produtividade nos rebanhos leiteiros. Desta forma, em conjunto com a seleção de animais mais eficientes no aproveitamento dos nutrientes, a longevidade dos animais é uma característica altamente desejável, e está diretamente relacionada a lucratividade do sistema. Segundo Drennan *et al.* (2008), as mensurações morfológicas realizadas nos animais são ferramentas importantes na avaliação do crescimento e desenvolvimento corporal e possuem alta correlação com o ganho de peso. As medidas corporais mais mencionadas na literatura para predizer o peso são o

perímetro torácico, o comprimento corporal, a altura da cernelha e da garupa e largura da garupa (Reis *et al.*, 2008).

Trabalhos que correlacionaram características de EA com características relativas ao tamanho esquelético (Basarab *et al.*, 2003; Nkrumah *et al.*, 2006; Smith *et al.*, 2010), não encontraram diferenças de altura na garupa em classes divergentes de CAR. Schenkel *et al.* (2004), relataram que o CAR e CA são independentes geneticamente da altura na garupa. Kelly *et al.* (2011), em estudo com 2.605 touros relataram correlação genética entre CAR e GPR com perímetro torácico de 0,11 e 0,23, respectivamente. Essas associações, no entanto, podem ser influenciadas pela raça, idade do animal no teste de eficiência, e interação genótipo x tipo de dieta (Berry e Crowley, 2013a,b).

Basarab *et al.* (2003) e Nkrumah *et al.* (2006) correlacionaram características de EA com características relativas ao tamanho corporal. Os animais considerados mais eficientes não apresentam características negativas quanto a sua morfologia. Esses autores não encontraram diferenças de altura na garupa em animais baixo, médio e alto CAR em animais cruzados e Angus.

Ceacero (2015) estimou alta herdabilidade para características de crescimento (altura e perímetro torácico). A herdabilidade para altura foi estimada em 0,61 enquanto para perímetro torácico foi de 0,31. Neste trabalho, o CMS foi altamente correlacionado geneticamente com altura e perímetro torácico (0,61 e 0,79, respectivamente), enquanto fenotipicamente, houve correlação moderada (0,45 para altura e 0,55 para perímetro torácico). Desta forma, o aumento da taxa de crescimento e do tamanho corporal do animal refletem em maior consumo de alimento.

A alta dependência do CMS com a taxa de crescimento e tamanho corporal podem ser eliminadas ou pelo menos reduzidas com a utilização de índices como CAR e de GPR (Ceacero, 2015). O grupo CAR obteve correlação genética baixa com tamanho corporal, a estimativa de correlação genética com altura foi próxima de zero (0,06) e perímetro torácico 0,14. Já o grupo GPR apresentou baixa e moderada correlação com altura e perímetro torácico (0,25 e 0,34, respectivamente), ou seja, a seleção de animais mais eficientes para GPR acarretará em aumento de estrutura corporal. Em geral, o autor encontrou correlações fenotípicas entre as características de EA com as características de crescimento e de carcaça foram baixas e próximas de zero.

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CAPÍTULO 2

INTERPRETIVE SUMMARY

Phenotypically divergent classification of preweaned heifer calves for feed efficiency indexes and their correlations with heat production and thermography.

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Leão et al. Given that feed represents the largest cost in dairy systems, heritable indicators of feed efficiency – residual feed intake (RFI) and residual body weight gain (RG) – are used to identify more efficient animals (low RFI and high RG) and to determine if more efficient animals can be selected during the preweaning phase. This study provided evidence that heat production is an important component of RFI variation among dairy calves. Heat production measured by face mask method was significantly different between low and high-RFI calves. These findings may be useful for understanding the consequences of selective breeding for reduced-RFI cows.

RUNNING HEAD: CALORIMETRY AND THERMOGRAPHY IN CALVES

Phenotypically divergent classification of preweaned heifer calves for feed efficiency indexes and their correlations with heat production and thermography

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ABSTRACT

The aims of this study were to assess if there is phenotypical divergence for feed efficiency (**FE**) during the preweaning phase, if FE is correlated with heat production (**HP**) measured by the face mask method or by surface skin temperature via thermography, and whether these methods are applicable to preweaned calves. Holstein x Gyr heifer calves (n = 36, birth BW = 32.4 ± 6.6 kg) enrolled between 4th and 12th w of age were classified into two residual feed intake (**RFI**) and residual growth (**RG**) groups: high efficiency (**HE**; RFI, n = 10; and RG, n = 9), and low efficiency (**LE**; RFI , n = 10; and RG, n = 8). Calves were fed milk (6 L/d) and solid feed (95% starter and 5% chopped Tifton 85 hay, as-fed). Growth were monitored weekly and feed intake (milk and solid feed) daily, during the whole period. Gas exchanges (O_2 consumption and production of CO_2 and CH_4) were obtained using a face mask at 45 ± 5 d of age and HP was estimated. Maximum temperatures were measured at 7 sites with an infrared camera at 62 ± 7 d of age. There was divergence in RFI and RG. Respectively, HE and LE calves had RFI of -0.14 kg/d and 0.13 kg/d, and RG of 0.05 kg/d and -0.07 kg/d. Dry matter intake (**DMI**) was 15% lower in HE-RFI compared with LE-RFI, but there were no differences in average daily weight gain (**ADG**). Within the RG test, there were no differences in DMI or ADG. HE-RFI calves consumed less O_2 (L/d) and produced less CO_2 (L/d). Heart rate and HP were lower for HE-RFI calves compared with LE-RFI. RFI was correlated with HP (r = 0.48), O_2 consumption (r = 0.48), CO_2 production (r = 0.48), and heart rate (r = 0.40). There were no differences in HP and gas exchanges between RG groups. Methane production was null in both groups. Eye temperature measured by thermography was 0.5°C greater in HE-RG than LE-RG calves. Differences in skin temperature between HE and LE calves were not observed at the other sites. These results support the hypothesis that calves are divergent for RFI, RG and FE during preweaning and divergence tests are applicable during this phase. The face mask method described here is a useful tool for estimating differences in HP among phenotypically divergent RFI calves. Eye temperature measured by IRT may have potential to screen phenotypically divergent RG calves.

Key words: calorimetry, residual feed intake, residual gain, thermography

INTRODUCTION

In dairy farming, approximately 60% of the production costs are related to feed (Ho *et al.*, 2005). An alternative to reduce feeding costs and increase production efficiency would be

to improve efficiency of feed usage (Berry *et al.*, 2014). In addition to the economical aspect, greater efficiency results in reduced waste of natural resources and methane emissions (Waghorn and Hegarty, 2011), thus reducing environmental impacts.

Presently, residual feed intake (**RFI**) is the most used index of feed efficiency (Koch *et al.*, 1963). Residual feed intake is independent of growth rate and BW. It is defined as the difference between realized intake and predicted intake, using a linear regression of individual intake as a function of mean metabolic body weight ($\mathbf{BW^{0.75}}$) and ADG, where RFI is the residual term. Highly feed efficient animals present negative RFI (i.e., realized intake is smaller than predicted intake), whereas animals with low feed efficiency present positive RFI (i.e., realized intake is greater than predicted intake). In addition to RFI, other measurements of efficiency have been described. Residual body weight gain (**RG**) is similar to RFI, but instead of regressing feed intake on BW and ADG as for RFI, RG is obtained from the regression of ADG on feed intake and BW (Crowley *et al.*, 2010). Hence, RG is positively associated with growth rate, but it is not associated with feed intake (Berry and Crowley, 2013).

Holstein heifer calves selected according to varying RFI during the growth phase presented divergences in RFI during the first lactation, even though the divergences were reduced at that time (Macdonald *et al.*, 2014; Gilbert *et al.*, 2017). It has been observed that this variation in RFI has moderate heritability (h^2 = 0.45, Crowley *et al.*, 2010). Thus, the selection of low-RFI animals as replacements could be an interesting tool, given that the association between efficiency in early and adult life could reduce time and costs of feed efficiency research and increase the selection pressure for this trait. There are few studies, however, that have evaluated this trait during the preweaning phase.

Although the biological mechanisms controlling RFI have not been fully elucidated, differences in digestion, nutrient requirements, and biochemical efficiency of feed usage have been identified as important factors (Herd and Arthur, 2009). The greatest variation in feed efficiency is likely related to variation in energy expenditure (Paddock, 2010). Energy expenditure, also known as heat production, can be estimated using indirect calorimetry, which measures oxygen consumption and carbon dioxide and methane production by the animal (Paddock, 2010). Regulation of body temperature has also been identified as an important aspect of physiological variation that could contribute to feed conversion efficiency in dairy cows (Herd and Arthur, 2009). This is due to greater use of energy sources for production of metabolic heat in detriment of milk yield in animals with greater body

temperature (Britt *et al.*, 2003). The use of infrared thermography (**IRT**) is another method for measurement of surface (e.g. skin) temperature, it allows obtainment of measurements in a non-invasive manner (Montanholi *et al.*, 2008).

The objectives of this experiment were to evaluate if phenotypically divergent classification is observed in relation to RFI and RG of preweaned heifer calves, and to determine if measurements of heat production (estimated by indirect calorimetry with a face mask) and of surface body temperature (measured by IRT) could be applied to the selection of high-efficiency calves during the preweaning phase. Our hypothesis was that there is phenotypically divergent classification in preweaned calves and that high efficient animals produce less heat.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of Embrapa Dairy Cattle, Brazil (protocol no. 02/2014). The experiment was conducted at the Embrapa Dairy Cattle Experimental Farm, located in Coronel Pacheco, Minas Gerais, Brazil.

Calves, Housing, Management, and Treatments

Holstein x Gyr F1 crossbred heifer calves (n = 36; BW at birth = 32.4 ± 6.6 kg, mean ± SD) born during Spring (October to December) were enrolled in the experiment. Immediately after birth, the calves were removed from their dams, weighed, and had their umbilical cords immersed in 10% iodine solution. Colostrum (10% of birth BW; > 50 g of IgG/L) was fed within 6 h after birth. Passive transfer of immunity was assessed using total serum protein. Blood samples were collected via jugular venipuncture within 48 h after birth, centrifuged at 1,800 × g for 10 min at room temperature (22–25°C), and TSP (g/dL) was measured using a refractometer (Serum protein REF-301, Biocotek, Beilun, Ningbo, China).

Calves were housed in sand-bedded individual pens (1.25 x 1.75 m, tethered with 1.2 m-long chains), which were allocated in a barn with open sides and end-walls. Calves were fed 6 L of milk/d during the whole preweaning period, split into 2 equal meals offered at 0700 and 1400 h. Calves were fed transition milk until 3 d of age and whole milk from 4 d of age until weaning. Water and solid feed were offered in buckets for ad libitum intake (10% orts of solid feed) throughout the experimental period. Solid feed as-fed was composed of 95% starter (Soylac Rumen 20% Flocculated, Total Alimentos, Três Corações, Minas Gerais, Brazil) and 5% chopped Tifton 85 hay (Table 1). Growth were monitored weekly and feed

intake (milk and solid feed) daily, were monitored until d 82 of age, when all calves were abruptly weaned.

Handling and Health Measurements

At d 8, preventive oral treatment against coccidiosis (Baycox Ruminants, Bayer, Leverkursen, Germany) was performed, at the dose of 3 mL per 10 kg of BW. Health and fecal scores were monitored daily by trained farm staff. Fecal score was graded according to Larson *et al.* (1977), as follow: 1- normal (firm, well formed); 2 – soft (tending to become slurred); 3 - loose (moderate diarrhea, slurred feces) and 4 – watery (severe diarrhea, liquid feces). A heifer was considered to have diarrhea if fecal scores were 3 or 4. All the episodes of diarrhea were on the first two weeks of life. All heifers were dehorned on the second week of life.

Nutrient composition analyses

Milk was sampled twice a day into plastic vials containing bronopol at each feeding for analysis of total solids (**TS**), crude protein (**CP**), lactose and fat content. Milk component analysis was performed by spectrophotometry using Bentley 2000 (Bentley Instruments, Chaska, MN). Mean ± SD values were 12.55% ± 0.93 TS, 3.93% ± 1.06 fat, 3.13% ± 0.16 CP and 4.54% ± 0.19 lactose.

Samples of solid feed (hay and starter) and its orts were collected thrice per week, pooled into a weekly sample, and stored at −20°C until analysis. Feed samples were dried in a forced-ventilation oven at 55°C for 72 h, ground to 1 mm particle size in a Wiley Mill (model 3, Arthur H. Thomas Co., Philadelphia, PA), and analyzed for DM, CP, ether extract (**EE**), ash, calcium, and phosphorus according to AOAC International (1995). Both NDF and ADF were determined according to the methods of Van Soest *et al.* (1991; Table 1); Gross energy (**GE)** was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL). Organic matter content (**OM**) was estimated as OM = 100 – Ash (AOAC, 1995). Non-fiber carbohydrates content (**NFC**) were calculated using the equation proposed by Hall *et al.* (1999): NFC_{ap} = 100 - (CP + NDF_{ap} + EE + Ash).

Intake and Performance

Daily solid feed was calculated by subtracting the orts from the offered quantities. Feed and water were weighed using a portable scale (WH-A04, WeiHeng, China). Daily milk intake was calculated as the sum of the differences between offered and refused amounts at

morning and afternoon feedings. Solid and milk feed intake were measured between 4 and 12 wk of age. Calves were weighted at birth, at 3 d of age (i.e. when the experimental diet was first offered), and weekly thereafter. Calves were weighed before the morning milk-feeding.

Feed efficiency indexes

Residual feed intake and RG were calculated over 56 observation days. Intake and performance were evaluated from d 28 to d 82 of age. Total DMI was obtained from the sum of milk DMI and solid feed DMI (offered amount minus orts in a DM basis). Average daily weight gain was calculated as the linear regression coefficient of BW (PROC REG; SAS Inst. Inc., Cary, NC), composed of 9 BW measurements per calf at 7-d intervals, and metabolic body weight ($BW^{0.75}$) was calculated using the BW at 23 d of the test. Feed efficiency was measured using the relationship between mean daily DMI and ADG (Khan *et al.*, 2007).

Dry matter intake, $BW^{0.75}$, and ADG were used to estimate RFI and RG using linear regressions (Koch *et al.*, 1963), where RFI and RG were calculated as the difference between realized and predicted DMI and ADG, respectively, as follows:

$$Yj = \beta0 + \beta1(BW^{0.75}j) + \beta2(ADGj \text{ or } DMIj) + ej,$$

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.

Calves were classified into two RFI and RG groups: high efficiency (**HE;** RFI < 0.5 SD below the mean (n = 10) and RG > 0.5 SD above the mean (n = 9)), and low efficiency (**LE;** RFI > 0.5 SD above the mean (n = 10) and RG < 0.5 SD below the mean (n = 8)).

Heat production

Heat production was evaluated at 45 ± 5 d of age. Three or 4 calves were evaluated each day. Calves were weighed before the morning milk-feeding on the evaluation days, and the feed intake of the day prior to the first evaluation day was compared between groups. Calves were placed in a squeeze chute, the face mask was fitted, and the exchange of gases was evaluated for 30 min in two consecutive days, 3 h after the morning feeding, starting at 1000 h. The delivery of diets on the evaluation days was planned to allow measurements approximately at 3 h post-feeding.

Respiratory gas exchanges were measured using a face mask adapted to calves according to the methodology described by Oss *et al.* (2016). The face mask was

manufactured with a 5.2-L polyethylene bottle, containing a 16-cm opening which covered the calf's muzzle up to the nasal bone, and was adapted to fit a halter. A flexible plastic membrane with a central hole with diameter of 6 cm was adapted to the superior external opening of the mask for pressure-adjustment to the calf's snout and air sealing, thus blocking the movement of gases through this opening. Two flanges (25 mm length x 20 mm diameter) were adapted centrally to the external outer part of the mask for installation of 2 valves (non-rebreathing Era® Mask Valves, Australia) to guarantee entry of external air to the interior of the mask and to block the exit of exhaled air, in order to force all exhaled air to pass through the tubing that led to the flowmeter. A 50 mm flange and an adapter (40 mm screw flange) were plugged to the funnel-like extremity of the mask, where a flexible tubing with inner diameter of 20 mm (Kanaflex S/A Indústria de plásticos, São Paulo, Brazil) was attached to connect the face mask to a 100-L PVC container equipped with a plastic bag at its superior end, in order to guarantee sealing and adaptation to the calf's respiratory movements, thus avoiding respiratory effort and aiding to standardize the air flow to the analyzers. Another flexible tubing (Kanaflex 2" S/A plastic industry, São Paulo, Brazil) connected the plastic container to a mass flowmeter (FK500, 75-500 L/min, Sable Systems, Henderson, NV). The PVC recipient's exit tubing was attached to a T-shaped rigid PVC joint (800 mm long x 50 mm across), placed longitudinally to contribute to the homogeneity of the internal air.

The rate of air flow through the mask (standard temperature and pressure corrected, 100 L/min) was controlled and measured by a mass flow controller (Flow Kit 500H; Sable Systems International, Las Vegas, NV). The mass flow controller acquired sub-samples of air from the mask at 500 mL/min for analysis. Simultaneously, a positive pressure pump (B-pump, Sable Systems, Henderson, NV) acquired sub-samples of ambient air (baseline) at 500 mL/min. Face mask and the ambient air were continuously sampled through Bev-A-Line tubes to a gas switching system (RM- 8 Flow Multiplexer, Sable Systems, Henderson, NV) in order to deliver gas samples to the analyzer set via a diaphragm subsampling pump (SS-4 Sub-Sampler Pump) at 200 mL/min. Samples from the face mask and ambient air were collected at 1 min intervals over 20 min; ambient air samples were also collected 5 min before and 5 min after the 20 min measurement to establish baseline gas levels. Humidity levels of the samples were monitored by a humidity meter (RH-300; Sable Systems International, Las Vegas, NV, USA), prior to flowing through CO_2 (CA-10, Sable Systems, Henderson, NV), CH_4 (MA-10, Sable Systems, Henderson, NV) and O_2 (FC-10, Sable Systems, Henderson, NV) gas analyzers. All data were recorded by an automated data acquisition program

(Expedata; Sable Systems International, Las Vegas, NV, USA). Considering the flow rate and the concentration of gases in the ambient air and in the air exiting the face mask, the software calculates the exhaustion rates (L/min) of O_2 (**VO_2**) and CO_2 (**VCO_2**) and CH_4 (**VCH_4**) using the equations from Lighton (2008). Total daily consumption of gases (L/d) was calculated by extrapolation of per-minute data to daily data (multiplication by 1440; 60 min x 24 h).

The energy expenditure was calculated as the estimated heat production using the equation by Brouwer (1965); the production of urinary nitrogen was not considered in the calculations (Verstegen *et al.*, 1987). The respiratory coefficient was calculated as the fractional CO_2 production divided by O_2 consumption.

The average heart rate (**HR**) measurements were recorded every 60 s and averaged over the 30 min of face mask evaluation. The HR was recorded using Polar equine transmitter and monitor (Model RS800CX, Polar Electro Inc., Kempele, Finland). The transmitters were embedded in a 10 cm wide strap with a velcro latch placed around the calf's girth behind the shoulders. The negative electrode was positioned on the right side and the positive electrode on the opposite side of the calf's thorax, parallel to the left elbow. Conductivity gel was applied on the area around the electrodes for increased conductance. Oxygen pulse (**O_2P**, mL/ heart beat) was determined as the average oxygen consumption per min over the HR per min during the face mask evaluation period.

Infrared Thermography

Thermal images of the calves were taken with an infrared camera (FLIR T420, FLIR Systems, Inc., Wilsonville, OR, EUA) on d 62 (±7) at 0600 h, 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 20ºC, and emission value of 0.98, according to the manufacturer's recommendations for biological tissues and to values used by Montanholi *et al.* (2008) and Montanholi *et al.* (2009). All thermal images were obtained in a roofed area. The evaluated anatomical regions were eye, jaw, snout, right-side ribs, left-side flank, right front limb, and hind area. Calves were manually restrained during the evaluations, with no manipulation of the evaluated areas. The average ambient temperatures recorded during imaging evaluations ranged between 18 and 25°C (relative humidity between 59 and 99%). The generated images were processes and interpreted using the FLIR Tools 5.6 software (FLIR Systems, Wilsonville, OR, USA).

In order 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 (Gomes *et al.*, 2016). Only the maximum temperature within each delimited area was considered, in order 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).

Statistical Analyses

Data were analyzed using SAS version 9.0 (SAS Institute Inc., Cary, NC). Performance and DMI were evaluated as repeated measures using SAS proc MIXED. The statistical model included fixed effects of treatment, time (week), treatment x time interaction, and the covariates described below. The experimental weeks were included as REPEATED statement and calf was nested within treatment as random effect. Heat production variables were submitted to ANOVA. The comparison between means was done using the Least Square Means (LSMeans) test, where the significance level was set at 10% for all tests. Pearson correlation coefficients between the response variables and RFI and RG were obtained with proc CORR. Birth BW and TSP were used as covariates in all analyses, and ambient temperature, humidity and temperature-humidity index were used as covariates in IRT analyses; covariates were kept in the models when their effect was significant.

RESULTS AND DISCUSSION

Feed efficiency indexes

High and low feed efficient calves were identified, where RFI was -0.14 kg/d and 0.13 kg/d ($P < 0.01$) for HE and LE, respectively. High efficiency calves had lesser DMI compared with low efficiency calves ($P = 0.06$; Table 2). Paddock (2010) affirmed that previous studies (Arthur *et al.*, 2001a; Arthur *et al.*, 2001b; Lancaster *et al.*, 2005) have reported similar differences in DMI (15 to 22% lower for HE-RFI calves) and feed conversion rate (19 to 21% lower for HE-RFI calves) between RFI phenotypes. In our study, HE-RFI had feed efficiency 13% higher than LE-RFI ($P < 0.01$; Table 2). The correlation coefficient between DMI and RFI was 0.60 ($P < 0.01$), in agreement with Ceacero *et al.* (2016), who reported phenotypical correlation of 0.73 and genetic correlation of 0.68 between DMI and RFI.

Divergence was also observed for RG, which was -0.07 kg/d for LE-RG group and 0.05 kg/d for HE-RG group ($P < 0.01$; Table 2). Negative values for RG are deemed to indicate

more inefficient animals, whereas animals with positive RG values (i.e. animals growing faster than expected) are deemed to be more efficient (Berry and Crowley *et al.*, 2013). There was no difference in DMI between groups of HE-RG and LE-RG. Crowley *et al.* (2010) and Berry and Crowley (2012) reported null genetic correlations between RG and DMI in cattle of European breeds. Crowley *et al.* (2010) reported a genetic correlation between RG and increased growth rate of 0.70, but there were no differences in DMI between high and low phenotypic RG. Santana *et al.* (2014) estimated negative genetic correlations of -0.12 between RG and DMI in Nellore cattle, in agreement with the results presented here.

The experiment adopted the traditional feeding management of preweaned calves, which consisted in supplying restricted milk (6 L per day) and ad libitum starter. So, the variation in intake would be related to the difference in solid feed intake among the animals. The animals evaluated for HE-RFI and LE-RFI had the same intake of milk 838 g MS. High efficiency RFI had lower intake of solid feed, 543 g MS compared to LE-RFI, 798 g MS (P = 0.03). For different RG groups, there was no difference in milk intake, 835 g MS, and for solid feed intake, 628 g MS.

Average daily gain was similar between HE and LE calves during the RFI and RG test-period (0.97 ± 0.1 kg/d). A meta-analysis by Berry and Crowley (2013) reported an estimate of 0.02 for the genetic correlation between RFI and ADG. Santana *et al.* (2014) observed genetic and phenotypic correlations of 0.11 and 0.05, respectively, between RFI and ADG for Nellore cattle, as well as genetic and phenotypic correlations of 0.12 and 0.20, respectively, between RG and ADG, in agreement with Berry and Crowley (2013). Herd and Arthur (2009) demonstrated similar BW and ADG that supports the independence of RFI from production. Average daily gain was correlated with RG (r = 0.15; P = 0.03).

Birth BW and BW at the start of the test (wk 4) were similar between groups within RFI and RG tests (Table 2). Due to similar ADG at the end of the test period, BW was similar between HE and LE groups for RFI and RG evaluations (P > 0.05).

Heat Production and Gas Exchanges

Heat production (kcal/d/$BW^{0.75}$) was lower for HE-RFI animals compared with LE-RFI animals (P = 0.03, Table 4). Calves with LE-RFI lost 15.26% more energy in the form of heat production (kcal/d) than HE-RFI calves. Nkrumah *et al.* (2006) reported heat production 21 and 10% lower for high and medium efficiency RFI steers than for steers with low efficiency RFI, respectively.

Heart rates were greater (P = 0.03, Table 4) for LE-RFI group compared with HE-RFI group, which is similar to observations by Paddock (2010), who reported greater heart rates and oxygen pulse rates (P < 0.05) in heifers with low efficiency RFI compared with those high efficiency RFI. Paddock (2010) also estimated that energy expenditure (kcal/$BW^{0.75}$) to be 17.4% greater (P < 0.05) in low efficiency RFI heifers than in high efficiency RFI heifers. Likewise, Lancaster *et al.* (2005) found that growing heifers and bulls with low efficiency RFI had faster heart rates than those with high efficiency RFI. There was no difference in O_2 pulse rate for RFI and RG groups (P > 0.05, Table 4) in our study, differing from results reported by Paddock (2010).

Animals of HE-RFI had lower O_2 consumption (L/ d) and lower CO_2 production expressed in L/d (P = 0.03) in comparison with the LE-RFI group. There were no differences in heat production and gas exchanges between RG groups (P > 0.05; Table 4). Correlations were observed between RFI and heat production (r = 0.48, P < 0.01), RFI and O_2 consumption (0.48, P < 0.01), RFI and CO_2 production (0.48, P < 0.01), and RFI and heart rate (0.40, P = 0.01) (Table 5). Methane production was null in all groups.

Cows that convert feed gross energy to net energy more efficiently or that have lower than expected maintenance requirements based on BW use less feed than expected and are classified as negative RFI; thus, such cows are considered high-efficiency animals. Cows classified as HE-RFI are likely digest and metabolize nutrients more efficiently and should have an overall greater efficiency and profitability if they are also healthy, fertile, and produce at a high multiple of maintenance (VandeHaar *et al.*, 2016). In the present experiment, we have observed similar characteristics for heifer calves, where HE-RFI calves converted smaller quantities of feed more efficiently, consequently producing a smaller amount of heat. A lower caloric increment of feeding is expected in animals that have reduced intake and similar performance (Herd and Arthur, 2009).

According to Nkrumah *et al.* (2006), the identification of metabolic and physiological reasons underlying the variation in feed efficiency in cattle that have similar BW and growth rate is a well-recognized prerequisite for the effective planning of breeding strategies to select more efficient animals. In their study, several potential metabolic and physiological pathways that could influence feed efficiency were considered. These included pathways that were generally related to variations in the efficiency of conversion of gross energy into metabolizable energy (because of differences in digestibility, generation of gases during ruminal fermentation, absorption of nutrients, waste excretion, and heat production) and the

subsequent efficiency of metabolizable energy use for maintenance and growth. We suggest that more research on feed efficiency of calves is needed to improve the understanding of its variation in this category of cattle.

Nkrumah *et al.* (2006) also reported greater heat production in low versus HE-RFI steers, despite the lack of differences in gross energy intake, which might be attributable to variation in metabolic efficiency. Variation in energy expenditure associated with differences in size of visceral organs has been proposed by Barasab *et al.* (2003) as a significant contribution to differences in HP between animals that have different RFI. Residual feed intake is positively correlated with DMI (0.71; Paddock, 2010) and it has been demonstrated that an increased DMI in cattle is generally accompanied by significant increases in the size of visceral organs (Ferrell and Jenkins, 1998).

There were no differences in BW at birth, at 1 wk of age, at the start of RFI evaluation (4^{th} wk), or at the end of the experimental period (12^{th} wk). In addition, ADG was not different between groups during the entire period (Table 2). It has been reported that RFI is repeatable within animal at different rearing phases, as the selection of animals based on RFI during the growth phase was predictive of the RFI rank of lactating cows (Macdonald *et al.*, 2014) and of veal calves (Gilbert at al., 2017). Furthermore, differences in gene expression associated with protein turnover and associated heat production in metabolically active tissues such as the liver and the gastrointestinal tract have been hypothesized as an alternative for increased understanding of the molecular mechanisms leading to variations in energy expenditure in cattle of similar BW and ADG (Nkrumah *et al.*, 2006).

Infrared thermography

Montanholi *et al.* (2009) reported that less efficient animals have greater heat production and present greater body surface temperature than more efficient animals, which is not in agreement with our observations for LE-RG and HE-RG calves. Eye temperatures were significantly greater (0.5°C) in HE-RG calves compared with LE-RG calves ($P = 0.08$; Table 3). No other significant differences in skin temperature at any of the measured sites were noted between HE and LE groups. However, Martello *et al.* (2015) also observed that temperatures of the front measured by IRT were lower ($P < 0.01$) for low efficiency RFI cattle than for high efficiency RFI cattle, and that the greater skin temperature measured by IRT for animals in the high efficiency RFI group may be related to an improved efficiency of thermoregulatory mechanisms, as the rectal temperature remained lower in the high efficiency

RFI group. Those authors suggested that the greater skin temperature in the high efficiency group probably occurred due to an effect of heat dissipation on maintenance of lower body core temperature in high efficiency animals compared with the low efficiency RFI group.

Although the mean rectal temperature value was not indicative of caloric stress, the maximum rectal temperature of 39.8°C observed by Martello *et al.* (2015) is above the normal physiological condition for ruminants. In our study, the maximum rectal temperature was 39.1°C. Skin temperature reflects heat dissipation (Scharf *et al.*, 2010), whereas the ocular surface is representative of the core body temperature (Dunbar *et al.*, 2009). Correlations were observed between eye temperature measured by IRT and RG (0.40; $P = 0.02$), demonstrating that the same could be occurring with the calves in the present study.

Scientific understanding of the physiological phenotype of low and high efficiency calves is poor and further knowledge of the physiological differences between animals of different efficiencies is required to develop a suite of potential indices for improved selection of more efficient animals. The infrared thermography technique was not suitable for the investigation of our hypothesis and more studies with calves are needed to evaluate these results.

CONCLUSIONS

The feed efficiency divergence tests are applicable to preweaned calves, as divergence in RFI and RG were observed during this rearing phase. The face mask method described here is a useful tool for estimating heat production differences in calves that present divergence in phenotypic RFI. Eye temperatures measured by IRT may have a potential to screen phenotypically divergent RG calves.

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TABLES

Table 1. Nutrient composition of hay, starter, and total mixed ration (TMR; 95% starter and 5% hay) offered to preweaned heifer calves between 4th and 12th w of age

Nutrient composition (DM basis, %)	Hay	Starter	TMR[9]
DM[1]	90.3	89.3	89,3
CP[2]	13.6	22.2	21,8
Organic Matter	80.8	77.9	78.0
Ether extract	3.7	4.6	4,6
NFC[3]	16.7	59.5	57.3
NDF[4]	70.1	24.5	26,8
ADF[5]	33.3	9.9	11.0
GE[6] (Kcal/kg)	3928.0	3728.0	3738.0
Ca[7]	0.8	2.0	1.9
P[8]	0.3	0.5	0.5

[1]DM = dry matter;

[2]CP = crude protein;

[3]NFC = Non-fibrous carbohydrate;

[4]NDF = neutral detergent fiber;

[5]ADF = acid detergent fiber;

[6]GE = Gross Energy;

[7]Ca= Calcium;

[8]P = Phosphorus;

[9]TMR: Total mixed ration

Table 2. Performance and feed efficiency indexes of preweaned heifer calves (4th to 12th w of age) classified as high efficiency (HE) and low efficiency (LE) from calculation of residual feed intake (RFI) and residual weight gain (RG)

	RFI[1]			*P* value				RG[8]			*P* value			
	HE[2]	LE[3]						HE	LE					
Item	(n = 10)	(n = 10)	SEM	G[4]	W[5]	G x W[6]	COV (Birth BW)[7]	(n = 9)	(n = 8)	SEM	G	W	G x W	COV (Birth BW)
RFI (kg/d)	-0.14	0.13	0.04	<0.01	-	-	-	-	-	-	-	-	-	-
RG (kg/d)	-	-	-	-	-	-	-	0.05	-0.07	0.02	<0.01	-	-	-
Birth BW (kg)[9]	34.5	34.3	1.05	0.94	-	-	-	32.1	34.6	1.20	0.24	-	-	-
Start BW (kg)[10]	47.5	46.2	1.33	0.23	-	-	-	46.0	44.8	1.74	0.46	-	-	-
Final BW (kg)[11]	102.3	101.1	2.88	0.81	-	-	-	103.0	95.5	3.07	0.18	-	-	-
DMI (g)[12]	1480	1744	51.80	0.06	<0.01	0.94	0.04	1535	1594	52.50	0.68	<0.01	0.89	0.05
FE[13]	0.70	0.61	0.02	<0.01	<0.01	0.59	<0.01	0.68	0.63	0.022	0.15	<0.01	0.81	0.02
ADG (kg/d)[14]	0.98	0.98	0.03	0.98	<0.01	0.89	-	1.10	0.92	0.06	0.19	0.37	0.47	-

[1]RFI = residual feed intake;

[2]HE = high efficiency;

[3]LE = low efficiency;

[4]G = group;

[5]W = week;

[6]GxW = group by week interaction;

[7]COV Birth BW = birth body weight as covariate;

[8]RG = residual gain;

[9]Birth BW: birth body weight;

[10]Start BW = start body weight

[11]Final BW = final body weight;

[12]DMI = dry matter intake;

[13]FE = feed efficiency;

[14]ADG = average daily gain

Table 3. Ambient temperature and temperature of different body sites (°C) of preweaned dairy heifer calves (62 ± 7 d of age) classified as high efficiency (HE) and low efficiency (LE) from calculation of residual feed intake (RFI) and residual gain (RG) measured by infrared thermography

	RFI[1]			*P* value		RG[6]			*P* value	
Site	HE[2] (n = 10)	LE[3] (n = 10)	SEM	G[4]	THI[5]	HE (n = 9)	LE (n = 8)	SEM	G	THI
Eye	37.6	37.6	0.12	0.98	0.02	37.8	37.3	0.13	0.08	0.18
Cheek	34.9	35.1	0.23	0.47	< 0.01	34.8	34.6	0.30	0.65	< 0.01
Snout	34.0	34.5	0.45	0.54	< 0.01	34.3	34.1	0.56	0.83	< 0.01
Right rib	34.5	34.8	0.32	0.50	< 0.01	34.7	34.2	0.35	0.32	< 0.01
Left flank	34.2	34.2	0.30	0.96	< 0.01	34.3	33.9	0.31	0.33	< 0.01
Right front limb	33.6	33.4	0.25	0.71	0.20	33.3	33.1	0.30	0.64	< 0.03
Hind area	36.6	37.2	0.22	0.12	< 0.01	37.0	36.6	0.28	0.45	0.08

Ambient temperature	21.7	21.8	0.41	0.75	< 0.01	21.4	21.1	0.51	0.31	< 0.01

[1]RFI = residual feed intake;

[2]HE = high efficiency;

[3]LE = low efficiency;

[4]G = statistical difference between HE and LE;

[5]THI = temperature-humidity index;

[6]RG = residual gain

Table 4. Dry matter intake on evaluation day, heat production and gas exchange in preweaned dairy heifer calves (45 ± 5 d of age) classified as high efficiency (HE) and low efficiency (LE) by residual feed intake (RFI) and residual gain (RG)

	RFI[3]				RG[4]			
Traits (unit)	HE[1] (n = 10)	LE[2] (n = 10)	SEM	*P*	HE (n = 9)	LE (n = 8)	SEM	*P*
DMI (g/d)[5]	1085	1405	43.1	<0.01	1163	1233	47.3	0.61
BW (kg)[6]	62.7	64.0	1.03	0.55	66.4	56.4	1.63	0.01
VO_2 (L/min)[7]	0.46	0.55	0.01	0.03	0.50	0.47	0.02	0.59
VCO_2 (L/min)[8]	0.41	0.49	0.01	0.03	0.44	0.42	0.01	0.61
Respiratory Coefficient	0.89	0.89	0.01	0.73	0.89	0.89	0.01	0.92
VO_2 (L/d)	668.1	787.5	20.60	0.03	713.6	680.9	22.90	0.59
VCO_2 (L/d)	591.6	702.2	19.20	0.03	634.8	604.6	21.30	0.61
VO_2(mL/d/BW)	7.41	8.56	0.20	0.04	7.43	8.58	0.25	0.14
VCO_2 (mL/d/BW)	6.56	7.64	0.19	0.04	6.60	7.66	0.24	0.17
Heart Rate (beat/min)	110	119	1.87	0.03	116	122	2.37	0.36
O_2 pulse (mL/min)	4.28	4.63	0.15	0.36	4.21	3.91	0.15	0.42
Heat production (kcal/d)	3294	3887	102	0.03	3521	3358	114	0.60
Heat production (kcal/d/$BW^{0.75}$)	148	172	4.03	0.03	151	165	4.48	0.35

[1]HE = high efficiency;

[2]LE = low efficiency;

[3]RFI = residual feed intake;

[4]RG = residual gain;

[5]DMI = dry matter intake;

[6]BW = body weight;

[7]VO_2 = volume of O_2;

[8]VCO_2 = volume of CO_2

Table 5. Correlations between feed efficiency indexes (RFI and RG) and gas exchanges, heat production, and heart rate of preweaned dairy heifer calves (4th to 12th w of age)

	RFI[1]		RG[2]	
Traits (units)	R[3]	*P*	R	*P*
VO_2 (L/min)[4]	0.48	<0.01	-0.01	0.93
VCO_2 (L/min)[5]	0.48	<0.01	-0.01	0.95
VO_2 (L/d)	0.48	<0.01	-0.01	0.93
VCO_2 (L/d)	0.48	<0.01	-0.01	0.95
VO_2 (L/d/$BW^{0.75}$)	0.46	<0.01	-0.29	0.10
VCO_2 (L/d/$BW^{0.75}$)	0.46	<0.01	-0.27	0.13
Heart rate (beat/min)	0.40	0.01	-0.15	0.40
Heat production (kcal/d)	0.48	<0.01	-0.01	0.93
Heat production (kcal/d/$BW^{0.75}$)	0.48	<0.01	-0.23	0.20

[1]RFI = residual feed intake;

[2]RG = residual gain;

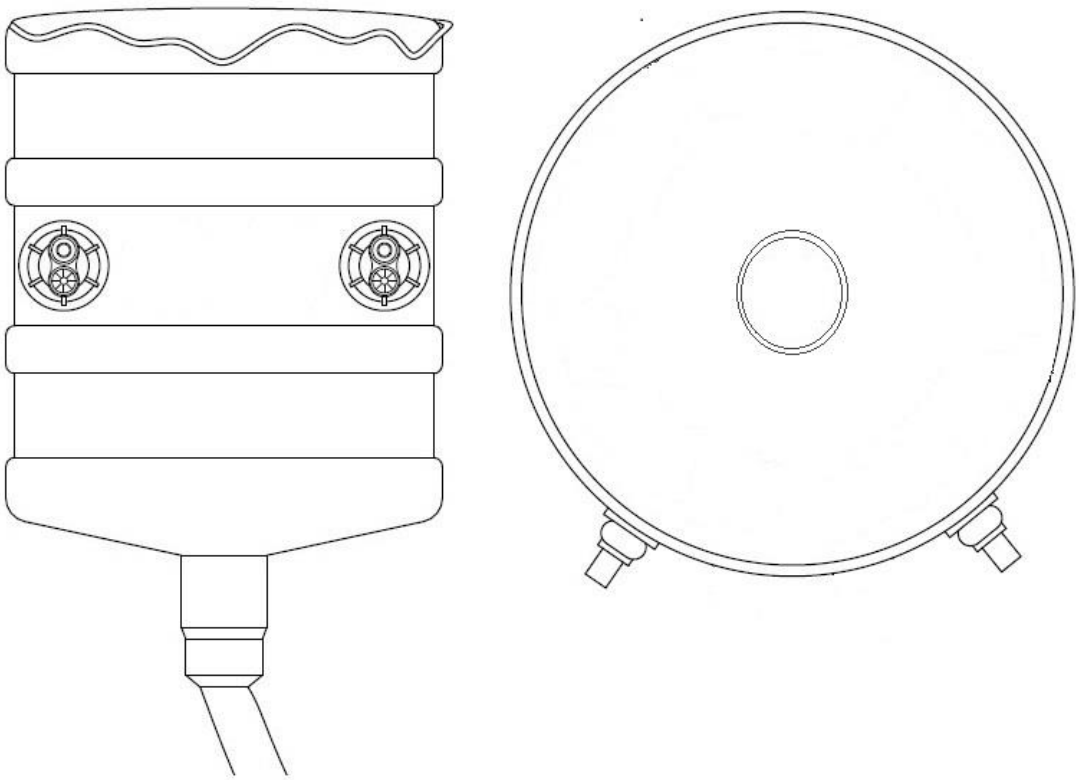
[3]R = correlation coefficient;

[4]VO_2 = volume of O_2;

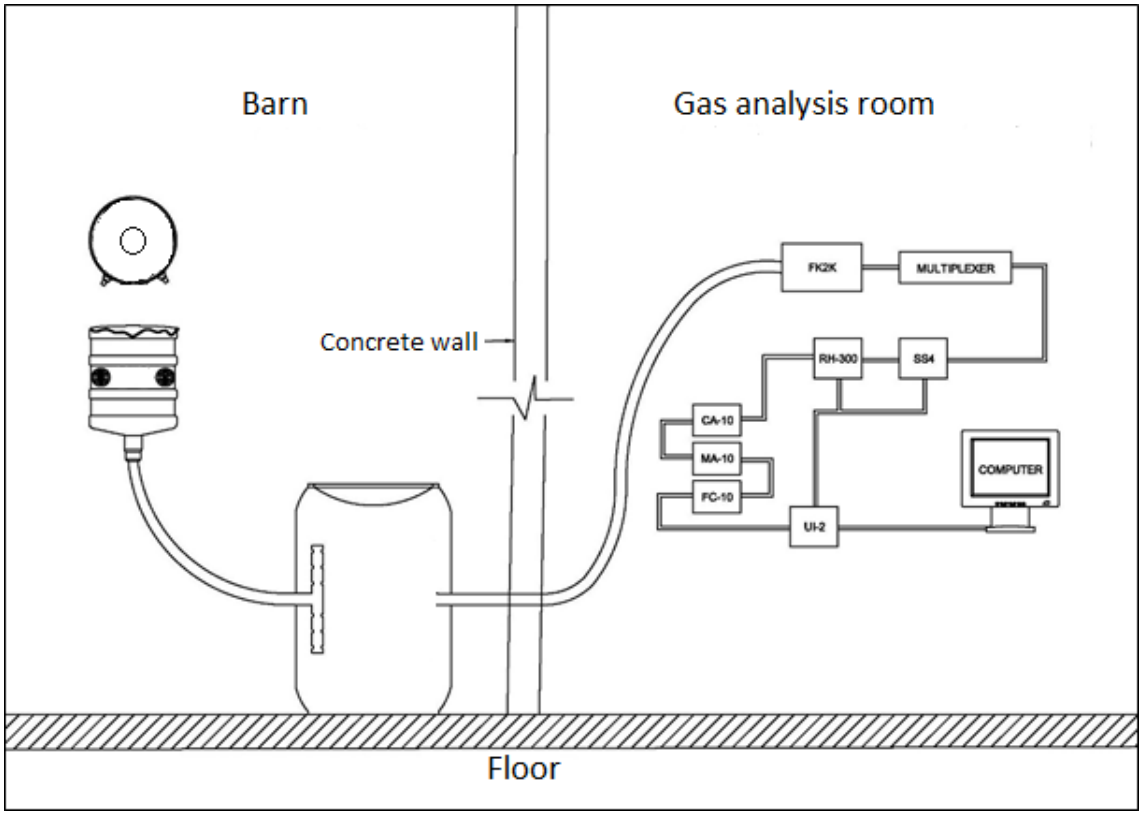
[5]VCO_2 = volume of CO_2

APPENDIX:

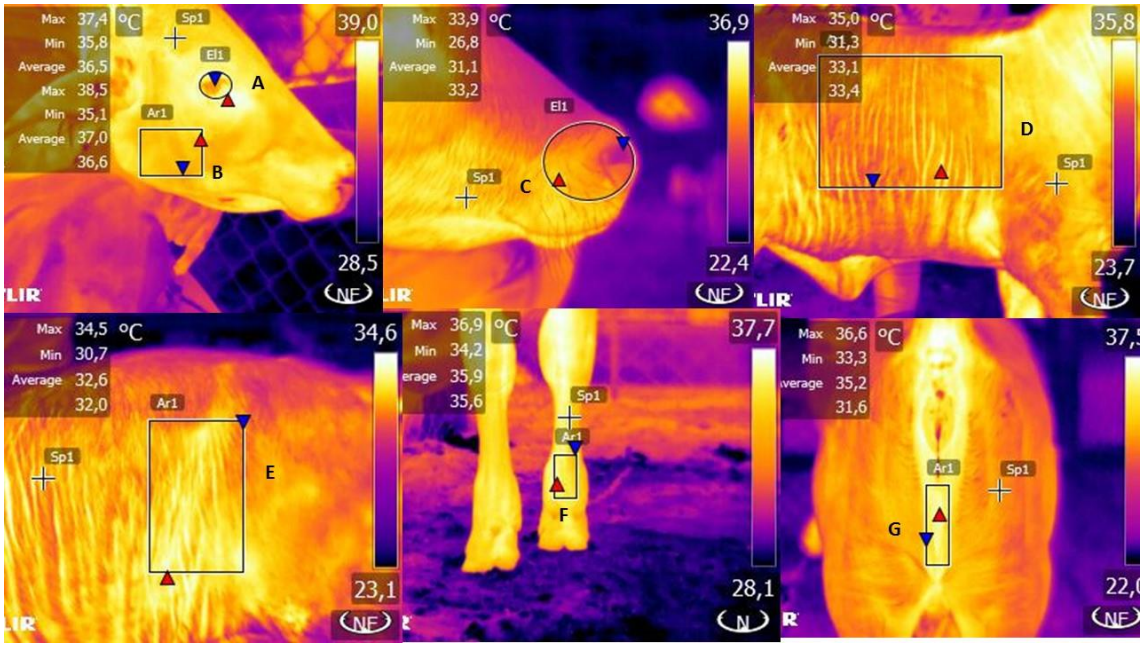
Leão. Figure 1. Calf fitted with the face mask



Leão. Figure 2. Face mask scheme (adapted from Sacramento, 2017), viewed from above on the left and view of the external opening on the right



Leão. Figure 3. Facilities (barn and gas analysis room) where the respiratory gas exchange tests were conducted (adapted from Sacramento, 2017).



Leão. Figure 4. Thermography sites

CAPÍTULO 3

INTERPRETIVE SUMMARY

Feed efficiency indexes and their correlations with body measurements and metabolites in blood and ruminal content of dairy heifers. *Leão et al.* Animal selection strategies need to focus on improving feed efficiency without compromising performance in order to increase the efficiency of cattle production. Since the variability in feed efficiency residual traits was acknowledged, there has been abundant research to assess its underlying physiological mechanisms. Every physiological step that affects the conversion of feed gross energy to animal product could be responsible for the variability in feed efficiency. For a better understanding of the contribution of the physiological processes in feed efficiency, ruminal parameters and associations between hormone concentrations and residual feed intake and residual body weight gain should be studied. This evaluation contributes to the characterization of individuals may assist in the identification of more efficient animal's, allowing understanding of physiological bases linked to the animal's response.

RUNNING HEAD: FEED EFFICIENCY MARKERS

Feed efficiency indexes and their correlations with body measurements and metabolites in blood and ruminal content of dairy heifers

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ABSTRACT

The aim of this study was to evaluate feed efficiency indexes and their correlations with body measurements and blood and ruminal metabolites of preweaned crossbred Holstein-Gyr heifer calves (n = 36, birth body weight = 32.4 ± 6.6 kg). Animals were enrolled between 4 and 12 wk of age and were classified into two residual feed intake (**RFI**) and residual body weight gain (**RG**) groups: High efficiency (**HE**; RFI, n = 10; and RG, n = 9), and low efficiency (**LE**; RFI, n = 10; and RG, n = 8). Calves were fed whole milk (6 L/d) and solid feed ad libitum (95% starter and 5% chopped Tifton 85 hay, as-fed). Blood samples were collected at 12 wk old and analyzed for glucose, insulin and β-hydroxybutyrate (**BHB**). Samples of ruminal content were collected on the same day and analyzed for pH and acetic, butyric, propionic, and total volatile fatty acids (**VFA**). Respectively, HE and LE calves had RFI of -0.14 kg/d and 0.13 kg/d, and RG of 0.05 kg/d and -0.07 kg/d. Dry matter intake (**DMI**) was 15% lower in HE-RFI compared with LE-RFI, but average daily gain (**ADG**) was not different among these groups. Within RG groups, there were no differences in DMI or ADG. Ruminal pH, N-NH_3 concentration, proportion of VFA, and molar proportions of propionic and acetic acids were not different between RFI or RG classes. Butyric acid proportion was similar among RFI groups, but tended to be greater for HE-RG than LE-RG. No differences in glucose, insulin, and insulin to glucose ratio were observed between groups. Glucose, insulin, and BHB were not different among RFI groups, but tended to be greater in HE-RG animals. Among growth characteristics, only initial hip width differed between RFI groups and withers height between RG groups. Overall, correlation coefficients between potential blood, rumen, or morphometric markers were low and generally not different from zero. Measurements of such metabolic indicators could be applicable towards early identification of feed efficient animals during the preweaning phase.

Key words: glucose, insulin, residual body weight gain, residual feed intake, volatile fatty acid

INTRODUCTION

Improvements in dairy cattle feed efficiency have substantial effects on economic efficiency and reduction of environmental impacts through lower feeding costs and emissions associated with dairy farming. In order to increase the biological and economic efficiencies of cattle production, animal selection strategies need to focus on improving feed efficiency without compromising performance.

According to Okine *et al.* (2004), a 5% improvement in feed efficiency has an economic impact eight times greater than a 5% increase in ADG. In addition, breeding for improved residual feed intake (**RFI**) can enhance feed efficiency without increasing the animal's mature size (Herd and Bishop, 2000). This has obvious positive ramifications to improved feed efficiency of growing and adult cattle. However, there is limited published information on phenotypic RFI and residual body weight gain (**RG**) of dairy heifers, as well as on its potential impacts on milk production and the biological factors contributing to variation in these traits.

Residual feed intake is the most used index of feed efficiency (Koch *et al.*, 1963), and it is defined as the difference between realized and predicted intake, using a linear regression of individual intake as a function of mean metabolic body weight ($\mathbf{BW^{0.75}}$) and ADG. This index is independent of growth rate and BW. Another measurement of efficiency is RG, which is similar to RFI, but instead of regressing feed intake on BW and ADG as for RFI, RG is obtained from the regression of ADG on feed intake and BW (Crowley *et al.*, 2010).

Since the variability in RFI has been acknowledged, there has been abundant research to assess its underlying physiological mechanisms (Herd *et al.*, 2004, Herd and Arthur, 2009). Evidence shows that no single physiological mechanism is responsible for the observed variability (Herd *et al.*, 2004). Theoretically, every physiological step that affects the conversion of feed gross energy to animal product could be associated with the observed variability in RFI.

Associations between hormone concentrations and RFI have been studied (Kelly *et al.*, 2010a; Nascimento *et al.*, 2015), and blood parameters may assist in the identification of differences in efficiency of feed utilization and in understanding the physiological bases linked to the animal's metabolic response, thus helping to identify more efficient animals. Kelly *et al.* (2010a) reported that circulating blood metabolites in growing heifers contributed with approximately 35% of the variation in RFI. Likewise, studies were done to evaluate differences in ruminal parameters between high and low RFI animals. Although evidence of differences in rumen digestion between these groups exists, results are contradictory and not conclusive (Lawrence *et al.*, 2011, 2013, Fitzsimons *et al.*, 2013, 2014). However, there is limited published information on the repeatability of RFI and its associated traits at different phases of the production cycle, which is ultimately essential for wide-scale adoption by producers.

Therefore, the objectives of this study were: 1) to evaluate feed efficiency indexes and their relationships with body measurements and blood and ruminal metabolites in preweaning period; 2) to determine if such measurements can be used as feed efficiency markers during the preweaning period. Our hypothesis was that there are differences in RFI and RG that explain better feed utilization by some animals and that such measurements can be used as feed efficiency markers.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of Embrapa Dairy Cattle, Brazil (protocol no. 02/2014). The experiment was conducted at the Embrapa Dairy Cattle Experimental Farm, located in Coronel Pacheco, Minas Gerais, Brazil.

Animals, Housing, Management, and Treatments

Holstein x Gyr F1 crossbred heifer calves were selected from a previous experiment (Leão *et al.*, 2018) in which RFI was determined for a group of heifers (n = 36; BW at birth = 32.4 ± 6.6 kg, mean ± SD). Immediately after birth, the calves were removed from their dams, weighed, and had their umbilical cords immersed in 10% iodine solution. Colostrum was fed within 6 h after birth (10% of birth BW; > 50 g of IgG/L). Passive immunity transfer was assessed using total serum protein. Blood samples were collected via jugular venipuncture within 48 h after birth, centrifuged at

1,800 × g for 10 min at room temperature (22–25°C), and total serum protein was measured using a refractometer (Serum protein REF-301, Biocotek, Beilun, Ningbo, China).

Heifers were housed in sand-bedded individual pens (1.25 x 1.75 m, tethered with 1.2 m-long chains), which were allocated in a barn with open sides and end-walls. Transition milk was fed until 3 d of age, followed by whole milk thereafter until abrupt weaning at 82 d of age. The volume fed during the whole preweaning period was 6 L of milk/d, divided into 2 equal meals offered at 0700 and 1400 h. Water and solid feed were offered in buckets for ad libitum intake (10% orts of solid feed) throughout the experimental period. Solid feed (as-fed) was composed of 95% starter (Soylac Rumen 20% Flocculated, Total Alimentos, Três Corações, Brazil) and 5% chopped Tifton 85 hay (Table 1). Growth was monitored weekly and intake of milk and solid feed were measured daily until weaning.

Handling and Health Measurements

At d 8, all calves received a preventive oral treatment against coccidiosis (Baycox Ruminants, Bayer, Leverkursen, Germany; 3 mL per 10 kg of BW). Trained farm staff monitored health and fecal scores daily. Fecal score was graded according to Larson *et al.* (1977), as follows: 1 – normal (firm, well-formed); 2 – soft (tending to become slurred); 3 - loose (moderate diarrhea, slurred feces) and 4 – watery (severe diarrhea, liquid feces). A heifer was considered to have diarrhea if fecal scores were 3 or 4 and was treated following the farm protocol. All diarrhea episodes occurred on the first two wk of age. All heifers were dehorned at 2 wk old. All episodes occurred on the first 3 wk, which did not influence the calculation of feed efficiency indexes.

Nutrient Composition Analyses

Milk was sampled twice a day at each feeding into plastic vials containing bronopol for analysis of total solids, crude protein, fat, and lactose content by spectrophotometry using Bentley 2000 (Bentley Instruments, Chaska, MN). Results of sampled milk were (means ± SD) 12.55% ± 0.93 TS, 3.13% ± 0.16 CP, 3.93% ± 1.06 fat and 4.54% ± 0.19 lactose.

Samples of solid feed (hay and starter) and its orts were collected thrice per wk, pooled into a weekly sample, and stored at −20°C until analysis. Feed samples were

dried in a forced-ventilation oven at 55°C for 72 h, ground to 1-mm particle size in a Wiley Mill (model 3, Arthur H. Thomas Co., Philadelphia, PA), and analyzed for DM, CP, ether extract (**EE**), ash, calcium, and phosphorus according to AOAC International (1995). Both NDF and ADF were determined according to the methods of Van Soest *et al.* (1991; Table 1). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL). Organic matter content was estimated as OM = 100 – Ash (AOAC, 1995). Non-fiber carbohydrates content was calculated using the equation proposed by Hall *et al.* (1999): $NFC_{ap} = 100 - (CP + NDF_{ap} + EE + Ash)$.

Intake and Performance

Milk, solid feed, and water intake were measured between 4 and 12 wk of age. Feed and water were weighed using a portable scale (WH-A04, WeiHeng, China). Daily solid feed and water intake were calculated by subtracting the orts weight from the total solid feed and water provided, respectively. Daily milk intake was calculated as the sum of the differences between offered and refused amounts at morning and afternoon feedings. Heifers were weighted at birth, at 3 d of age (i.e. when the experimental diet was first offered), and weekly thereafter, always before the morning milk feeding.

Feed Efficiency Indexes

Residual feed intake and residual gain were calculated over 56 d of observation. Intake and performance were evaluated from d 25 to d 80 of age. Total DMI was obtained from the sum of milk DMI and solid feed DMI (offered amount minus orts on DM basis). Average daily gain was calculated as the linear regression coefficient of BW (PROC REG; SAS Inst. Inc., Cary, NC), composed of nine BW measurements per heifer at 7-d intervals. Metabolic body weight ($BW^{0.75}$) was calculated using the BW at the 23rd d of the test. Feed efficiency was measured using the relationship between mean daily DMI and ADG (Khan *et al.*, 2007).

Linear regressions were used to estimate RFI and RG from (Koch *et al.*, 1963), where RFI and RG were calculated as the difference between realized 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 Y_j is the standardized DMI (RFI) or ADG (RG) of heifer j, β_0 is the intercept, β_1 is the regression coefficient for $BW^{0.75}$, β_2 is the regression coefficient for ADG (RFI) or DMI (RG), and e_j is the error term for heifer j.

Heifers were classified into two RFI and RG groups: high efficiency [**HE;** RFI <0.5 SD below the mean (n = 10) and RG >0.5 SD above the mean (n = 9)], and low efficiency [**LE;** RFI >0.5 SD above the mean (n = 10) and RG <0.5 SD below the mean (n = 8)].

Blood Collection and Analyses

Blood samples were obtained by jugular puncture from each animal 3 h after the morning milk feeding on the same day of ruminal fluid collection (12^{th} wk) during the RFI and RG evaluation period. Samples collected into untreated tubes were used for dosage of insulin and BHB and those from sodium fluoride-treated tubes for were used to determine glucose concentration (Vacutainer, Becton Dickinson, São Paulo, Brazil). Tubes were placed in crushed ice until centrifugation at 1,800 × g for 10 min at room temperature (22–25°C). Plasma or serum aliquots were stored at −20°C until analysis. Plasma glucose was measured on a microplate Spectrophotometer EON (Biotek Instruments Inc., Winooski, VT) using an enzymatic colorimetric method (Kovalent do Brasil Ltda., Rio de Janeiro, Brazil). Serum insulin was analyzed using a bovine ELISA kit (Mercodia, Uppsala, Sweden) and serum BHB was determined using an enzymatic kinetic kit RANBUT - Ref.: RB 1007 (RANDOX Laboratories - Life Sciences Ltd, Crumlin, UK) and spectrophotometry (Automatic System for Biochemistry, Model BIOPLUS BIO 2000®).

Rumen Variables and Analyses

Ruminal fluid samples were obtained from each animal at the end of the experimental period (12^{th} wk). Samples of approximately 50 mL were collected using a stomach tube technique 3 h after milk feeding (Lodge-Ivey *et al.*, 2009; Henderson *et al.*, 2013; Kumar *et al.*, 2015). Ruminal fluid pH was measured immediately after collection using a portable potentiometer (DM-2-Digimed, São Paulo, Brazil). Rumen content samples (5 mL) were acidified with 1 mL of sulfuric acid (500 mL/L) and stored at -20°C until analysis of ruminal $N\text{-}NH_3$ concentration, which was quantified after distillation of Kjeldahl with magnesium oxide and calcium chloride according to

method 920.03 (AOAC, 1980). Samples were centrifuged at 1,800 × g for 10 min at room temperature (22-25° C) for measurement of VFA concentration by high performance liquid chromatography (Waters Alliance e2695 Chromatograph, Waters Technologies do Brazil LTDA, Barueri, Brazil).

Morphometric Measurements

Morphometric measurements were performed weekly (between wk 4 and 12) before morning milk feeding and after weighing, in a flat location that allowed the animals to remain with their limbs well set. Withers height (distance from the withers to the ground) and hip height (distance from ileosacral tuberosity to the ground) were measured using a hipometer (Walmur, Porto Alegre, Brazil). Hip width (distance between the two iliac tuberosities), and heart girth (measured immediately caudally to the front limbs) were evaluated with a measuring tape (Bovitec, São Paulo, Brazil). The variation of each body measurement was calculated as the difference between the final (12^{th} wk) and initial (4^{th} wk) values.

Statistical Analyses

Data were analyzed using SAS version 9.0 (SAS Institute Inc., Cary, NC). Performance, water intake, and DMI were evaluated as repeated measures using SAS proc MIXED. The statistical model included fixed effects of treatment, time (wk), treatment x time interaction, and birth BW and total serum protein as covariates when their effect in the model was significant. Experimental wk was included in the REPEATED statement and heifer was nested within treatment as random effect. Blood, rumen and morphometric variables were submitted to ANOVA. The comparison between means was done using the Least Square Means test, where the significance level was set at 5%. Tendencies were accepted when $P \leq 0.10$. Pearson correlation coefficients between the response variables and RFI and RG were obtained with proc CORR.

RESULTS AND DISCUSSION

Feed Efficiency Indexes

Residual feed intake ranged from −0.14 to 0.13 kg/d ($P < 0.01$) for HE and LE, respectively, equating to a difference of 0.27 kg of DMI/ d between the groups ranked

high and low RFI. Residual weight gain ranged from 0.05 to −0.07 ($P < 0.01$), equating to a difference of 0.12 kg of ADG between the groups ranked high and low efficiency for RG. At the start of the experimental period (4^{th} wk), BW ± SD was 46.78 ± 5.81 kg in the RFI test and 45.36 ± 6.98 kg in the RG test. At the end of experimental period (12^{th} wk), BW was 101.68 ± 12.89 kg and 99.49 ± 12.68 kg for RFI and RG tests, respectively.

The effect of phenotypic classification by RFI and RG on feed intake, feed efficiency, and performance is shown in Table 2. Animals classified as HE-RFI and LE-RFI had similar milk intake (838 g DM/d) but HE-RFI had lower solid feed intake compared to LE-RFI (543 vs. 798 g DM/d, respectively; $P = 0.03$). Intake of milk and solid feed were not different among RG groups (835 g and 628 g DM/d, respectively). Animals in HE-RFI consumed 15% less feed than their counterparts ranked as LE-RFI ($P < 0.01$), similarly to growing beef heifers RFI results by Kelly *et al.* (2010a).

This is an important study evaluating feed efficiency divergence in preweaned dairy heifers, an analysis that is complicated by the large difference in milk and starter nutritional values and digestion. In order to produce results that are useful for the dairy industry, we designed an experiment following the feeding practices adopted in commercial dairies (i.e., set amount of milk and ad libitum starter). Although this practice represents common rearing conditions, it limits the understanding of the separate effects of milk and starter. Therefore, it is not possible to determine if the variation observed for certain variables results from milk or starter intake, neither is it possible to study the interactions among them. However, as milk supply was fixed at 6 L/d for all animals during the trial, DMI variation among heifers would be related to the difference in solid feed intake. Individual daily milk and starter intake were accurately measured.

Body weight at birth, start and end of experiment, as well as ADG did not differ between RFI or RG groups. Considering that only solid feed intake influenced the differences between the groups, and that during most of the preweaning period the consumption of solid feed is small, we can hypothesize that the differences between HE and LE groups were not enough to guarantee differences in ADG, especially in the evaluation of RG.

The HE-RFI group presented feed efficiency 13% greater than LE-RFI ($P < 0.01$). The correlation coefficient between DMI and RFI was 0.60 ($P < 0.01$), in agreement with Kelly *et al.* (2010a), who reported a correlation coefficient of 0.47. Likewise, Arthur *et al.* (2001a,b) and Nkrumah *et al.* (2007a), studying diverse breeds of cattle, reported positive phenotypic correlations ranging from 0.60 to 0.72 between RFI and DMI, indicating that selection of more favorable RFI phenotypes should result in significant reductions in feed intake.

By design, phenotypic correlations between RFI and ADG are unsurprisingly near to zero. Residual feed intake is genetically independent of growth and body size in growing bulls (Arthur *et al.*, 2001 a,b) and steers (Nkrumah *et al.*, 2004), in agreement with our observations.

There was a tendency ($P = 0.06$) for greater water intake by LE-RFI heifers, which consumed 1.0 L more per day than HE-RFI, possibly due to the greater DMI observed in the LE group. Water intake per BW and per $BW^{0.75}$ were greater for LE-RFI ($P = 0.08$ and $P = 0.09$, respectively) and there was a strong correlation between DMI and water intake ($r = 0.82$, $P < 0.01$) for the RFI group. No water intake variable was different between RG groups and the correlation between DMI and water intake was of 0.65 ($P = 0.03$). As DMI did not differ among RG groups, there was also no effect on water intake.

Morphometric Measurements

Differences were not detected between groups in RFI test for withers height, hip height and heart girth ($P > 0.05$; Table 3) and between groups in RG test for hip height, hip width and heart girth ($P > 0.05$; Table 3). Among RFI groups, HE-RFI had greater initial hip width than LE-RFI ($P = 0.03$), but the final measure and the variation did not differ, probably because LE-RFI had greater DMI resulting in similar size to HE-RFI at the end of the experimental period. Lawrence *et al.* (2011) observed similar results, where no differences among RFI groups were detected in morphometric measurements. Others have indicated that morphological traits do not differ according to RFI ranking, consequently not altering the morphometric pattern of the animals selected for RFI (Basarab *et al.*, 2003; Nkrumah *et al.*, 2004; Kelly *et al.*, 2010a).

The variation in withers height was greater for HE-RG than LE-RG (P = 0.01), demonstrating greater growth in the former group. These results are similar to observations by Crowley *et al.* (2010), who reported correlations between RG and growth rate of 0.70.

Blood Metabolites

No significant differences in glucose and insulin concentrations were observed among groups of RFI and RG tests at the 12th wk, but the glucose:insulin ratio tended (P = 0.07) to be lower in the HE-RG group (Table 4). The concentrations of blood metabolites shown in Table 4 are within the normal range reported in the literature for 12-wk old heifers.

Nascimento *et al.* (2015), studying growing Nellore cattle, did not observe differences in glucose concentration between RFI groups, but HE-RFI animals had lower glucose:insulin ratio and higher insulin concentrations than LE-RFI animals. These authors hypothesized that HE-RFI animals have greater satiety due to the greater blood insulin concentration. Kelly *et al.* (2011) also observed greater insulin concentrations in HE-RFI beef bulls, when evaluating associations among body measurements and blood variables.

In the present study, glucose metabolism was not different among RFI-divergent animals. However, HE-RG heifers had lower glucose:insulin ratio, indicating greater secretion of insulin per unit of blood glucose by these animals. Insulin is an important hormonal regulator of metabolism and inhibitor of hepatic gluconeogenesis by reducing hepatic absorption of some glucose precursors and directing the flow of glycogenic nutrients to muscle and adipose tissues (Brockman and Laarveld, 1986). Greater insulin concentration promotes protein and lipid synthesis and body weight gain, which may justify the greater withers height variation in the HE-RG group (Table 3).

Twelve-wk old heifers classified as HE-RG had lower BHB concentration (P = 0.01), but this metabolite was not different among RFI groups. In our study, DMI did not differ among HE-RG and LE-RG, but HE-RG tended to have greater ruminal butyrate concentration (Table 5). In the young animal, BHB is produced when rumen butyrate is metabolized by the rumen epithelial cells. As blood and rumen samples were

collected simultaneously, HE-RG animals could have lower butyrate absorption and therefore lower BHB blood concentration at that time.

There were no significant correlations between blood variables (glucose, insulin, glucose:insulin ratio, and BHB) and RFI, DMI, and feed efficiency. Significant correlations were observed between blood insulin and glucose concentrations for RFI (r = 0.46; P = 0.05) and RG groups (r = 0.61; P = 0.02). There were negative correlations of -0.54 (P = 0.03) between RG and BHB and -0.68 (P = 0.01) between RG and glucose:insulin ratio. Nascimento *et al.* (2015) reported no significant correlations between glucose, insulin and glucose:insulin ratio and $BW^{0.75}$, ADG, DMI, and RFI. Significant correlations were only observed between RFI and blood insulin concentration (r = -0.23; $P < 0.05$) and glucose:insulin ratio (r = 0.30; $P < 0.01$).

Rumen Variables

Residual feed intake and RG had no effect ($P > 0.05$) on rumen fermentation (Table 5), except for $N\text{-}NH_3$ concentration, which was greater for HE-RG heifers. Rumen fermentation variables was not correlated with the feed efficiency indexes. According to Alende *et al.* (2016), there are evidences of differences in rumen digestion between RFI-divergent cattle. However, results are contradictory and not conclusive (Lawrence et al, 2011, 2013; Fitzsimons et al, 2013, 2014).

Fitzsimons *et al.* (2014), using a high concentrate diet (rolled barley 860 g/kg DM) reported no differences in rumen pH and VFA proportions. However, a previous study using a diet comprised purely of grass silage (Fitzsimons *et al.*, 2013) reported that HE-RFI cattle tended to have greater ruminal propionate concentration and lower acetate:propionate ratio. This is in agreement with results reported by Lawrence *et al.* (2011, 2013), also feeding high fiber diets. Lower acetate:propionate ratio in HE-RFI cattle is consistent with greater energy efficiency and lower methane production. It seems, therefore, that differences in rumen fermentation profile are evident in high fiber diets but not in high concentrate diets such as those fed to preweaning calves.

Concentration of $N\text{-}NH_3$ was greater in HE-RG calves, indicating that this group obtained adequate ruminal fermentation efficiency. In this group, rumen ammonia levels were similar to those cited by Van Soest (1982). On the other hand, the LE-RG group presented values below those considered optimal,

suggesting lower ruminal efficiency. This could be explained by individual variation, since DMI, ADG and solid feed were the same.

Fitzsimons *et al.* (2014) stated that differences in ruminal fermentation may also arise from differing microbial populations in HE and LE-RFI cattle. Carberry *et al.* (2012) showed that, although subject to an effect of diet type, RFI and rumen microbial diversity are associated in beef heifers. Similarly, in an earlier study, Guan *et al.* (2008) observed that specific bacterial groups may only inhabit the rumen of HE-RFI steers. These findings suggest that differences in ruminal fermentation variables may exist between animals of high and low efficiency for RFI and that these differences are potentially mediated by diet type. Further research examining factors regulating ruminal pH and VFA production between HE and LE-RFI groups is needed. The establishment of the relationship between RFI and ruminal fermentation is particularly pertinent for forage-based systems such as those of Brazilian dairies.

CONCLUSIONS

In conclusion, RFI and RG groups had no negative effects on relevant metabolic, morphometric, and traits such as morphometric measurements and metabolic and performance characteristics. Consequently, such feed efficiency divergence tests are applicable to selection of more efficient preweaned heifers. However, there was no evidence of strong associations between blood or rumen metabolites and RFI and RG tests. Thus, it is unlikely that measurements of metabolic indicators, *per se*, will be useful in the early identification of more efficient animals. Understanding the underlying physiological basis for improved feed efficiency in dairy heifers requires further investigations.

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TABLES

Table 1. Nutrient composition of hay, starter, and total mixed ration (TMR; 95% starter and 5% hay)

Nutrient composition (DM basis, %)	Hay	Starter	TMR[9]
DM[1]	90.3	89.3	89.3
CP[2]	13.6	22.2	21.8
Organic Matter	80.8	77.9	78.0
Ether extract	3.7	4.6	4.6
NFC[3]	16.7	59.5	57.3
NDF[4]	70.1	24.5	26.8
ADF[5]	33.3	9.9	11.0
GE[6] (Kcal/kg)	3928.0	3728.0	3738.0
Ca[7]	0.8	2.0	1.9
P[8]	0.3	0.5	0.5

[1]DM = dry matter;

[2]CP = crude protein;

[3]NFC = Non-fibrous carbohydrate;

[4]NDF = neutral detergent fiber;

[5]ADF = acid detergent fiber;

[6]GE = Gross Energy;

[7]Ca = Calcium;

[8]P = Phosphorus;

[9]TMR = Total mixed ration

Table 2. Performance and feed efficiency indexes of preweaned heifers (4 to 12 wk of age) classified as high efficiency (HE) and low efficiency (LE) according to residual feed intake (RFI) or residual weight gain (RG)

	RFI[1]			*P* value				RG[8]			*P* value			
Item	HE[2]	LE[3]	SEM	G[4]	W[5]	G x W[6]	COV (Birth BW)[7]	HE	LE	SEM	G	W	G x W	COV (Birth BW)
RFI (kg/d)	-0.14	0.13	0.04	<0.01	-	-	-	-	-	-	-	-	-	-
RG (kg/d)	-	-	-	-	-	-	-	0.05	-0.07	0.02	<0.01	-	-	-
Birth BW (kg)[9]	34.5	34.3	1.05	0.94	-	-	-	32.1	34.6	1.20	0.24	-	-	-
Start BW (kg)[10]	47.5	46.2	1.33	0.23	-	-	-	46.0	44.8	1.74	0.46	-	-	-
Final BW (kg)[11]	102.3	101.1	2.88	0.81	-	-	-	103.0	95.5	3.07	0.18	-	-	-
DMI (g)[12]	1480	1744	51.80	0.06	<0.01	0.94	0.04	1535	1594	52.50	0.68	<0.01	0.89	0.05
Milk consumption (g/d)	840	837	2.34	0.77	<0.01	0.33	-	838	831	2.98	0.50	0.01	0.92	-
Total solid diet consumption (g/d)	543	798	45.47	0.04	<0.01	0.83	-	599	660	46.05	0.64	<0.01	0.85	-
FE[13]	0.70	0.61	0.02	<0.01	<0.01	0.59	<0.01	0.68	0.63	0.02	0.15	<0.01	0.81	0.02
ADG (kg/d)[14]	0.98	0.98	0.03	0.98	<0.01	0.89	-	1.10	0.92	0.06	0.19	0.37	0.47	-
Water Intake (L/d)	2.4	3.4	0.17	0.06	<0.01	0.75	0.04	2.4	3.15	0.19	0.33	<0.01	0.85	-

[1]RFI = residual feed intake;

[2]HE = high efficiency;

[3]LE = low efficiency;

[4]G = group;

[5]W = week;

[6]GxW = group by week interaction;

[7]COV Birth BW = birth body weight as covariate;

[8]RG = residual gain;

[9]Birth BW: birth body weight;

[10]Start BW = start body weight

[11]Final BW = final body weight;

[12]DMI = dry matter intake;

[13]FE = feed efficiency;

[14]ADG = average daily gain

Table 3. Morphometric measurements of preweaned heifers (4 to 12 wk of age) classified as high efficiency (HE) and low efficiency (LE) according to residual feed intake (RFI) or residual weight gain (RG)

Item	RFI[3]				RG[4]			
	HE[1]	LE[2]	SEM	*P*	HE	LE	SEM	*P*
Withers height (cm)								
Initial	79.42	77.88	0.89	0.41	78.91	79.62	1.00	0.74
Final	94.87	93.20	0.61	0.18	94.05	92.25	0.87	0.31
Variation	15.45	15.11	4.02	0.77	15.14	12.62	0.54	0.01
Hip height (cm)								
Initial	83.25	82.00	0.99	0.51	83.27	83.37	1.07	0.97
Final	98.99	97.70	0.67	0.35	97.88	96.68	0.99	0.56
Variation	15.74	15.70	0.57	0.97	14.61	13.31	0.53	0.23
Heart girth (cm)								
Initial	81.15	79.45	0.77	0.28	79.11	80.56	1.02	0.50
Final	105.54	103.95	0.98	0.43	104.48	104.81	1.14	0.89
Variation	24.39	24.50	0.73	0.94	25.37	24.25	0.86	0.53

Hip width (cm)								
Initial	22.33	20.60	0.41	0.03	20.55	20.25	0.55	0.79
Final	28.60	28.60	0.56	1.00	29.00	28.50	0.46	0.60
Variation	8.50	8.00	1.10	0.83	8.44	8.25	0.44	0.83

[1]HE = high efficiency;

[2]LE = low efficiency;

[3]RFI = residual feed intake;

[4]RG = residual body weight gain;

Table 4. Hormones and metabolites of preweaned heifers (12 wk of age) classified as high efficiency (HE) and low efficiency (LE) according to residual feed intake (RFI) or residual weight gain (RG)

Item	RFI				RG			
	HE[2]	LE[3]	SEM	*P* value	HE	LE	SEM	*P* value
Glucose (mg/dL)	119.44	111.63	3.230	0.24	111.27	107.27	3.910	0.63
Insulin (μU/mL)	1.72	1.22	0.186	0.18	1.54	1.18	0.257	0.51
BHBA (mmol/L)	0.32	0.31	0.15	0.67	0.28	0.38	0.020	0.01
Glucose:Insulin ratio (mg/ μU)	0.80	1.17	0.255	0.20	0.83	1.69	0.346	0.07

[1]RFI = residual feed intake;

[2]HE = high efficiency;

[3]LE = low efficiency;

[6]RG = residual gain

Table 5. Ruminal parameters of preweaned dairy heifers (12 wk of age) classified as high efficiency (HE) and low efficiency (LE) according to residual feed intake (RFI) or residual weight gain (RG)

Traits	RFI[3]				RG[4]			
	HE[1]	LE[2]	SEM	*P*	HE	LE	SEM	*P*
N-NH_3 (mg/dL)	9.87	9.88	1.95	0.99	10.42	6.5	2.27	0.06
pH	5.97	6.24	0.15	0.39	6.11	5.67	0.17	0.21
VFA[5] (µmol/mL)	50.40	41.22	4.23	0.29	51.54	47.23	2.57	0.43
Acetate (µmol/mL)	23.94	20.15	1.93	0.34	21.57	23.14	1.56	0.63
Propionate (µmol/mL)	19.75	17.40	1.87	0.55	18.31	20.14	1.65	0.60
Butirate (µmol/mL)	3.99	3.32	0.34	0.35	3.99	3.05	0.28	0.10
Acetate:Propionate	1.25	1.20	0.04	0.58	1.19	1.19	0.04	0.95

[1]HE = high efficiency;

[2]LE = low efficiency;

[3]RFI = residual feed intake;

[4]RG = residual body weight gain;

[5]VFA = Volatile fatty acids