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ORIGINAL ARTICLE

Rumen fluid metabolomics of beef steers differing in feed efficiency

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Abstract

Introduction Beef is the most consumed red meat in the United States, and the US is the largest producer and consumer of beef cattle globally. Feed is one of the largest input costs for the beef cattle industry, accounting for 40–60% of the total input costs. Identifying methods for improving feed efficiency in beef cattle herds could result in decreased cost to both producers and consumers, as well as increased animal protein available for global consumption.

Methods In this study, rumen fluid was collected from low- (n = 14) and high-RFI (n = 15) steers. Rumen fluid was filtered through a 0.22 μM syringe filter, extracted using 0.1% formic acid in acetonitrile:water:methanol (2:2:1) and injected into the Dionex UltiMate 3000 UHPLC system with an Exactive Plus Orbitrap MS. Peaks were identified using MAVEN and analyzed using MetaboAnalyst 4.0 and SAS. Significance was determined using an $\alpha \leq 0.05$.

Results Eight metabolites were greater in low-RFI steers compared to high-RFI steers, including 3,4-dihydroxyphenylacetate, 4-pyridoxate, citraconate, hypoxanthine, succinate/methylmalonate, thymine, uracil, and xylose ($P \leq 0.05$). These metabolites were predominantly involved in amino acid and lipid metabolism.

Conclusions Rumen fluid metabolomes differ in steers of varying feed efficiencies. These metabolites may be used as biomarkers of feed efficiency, and may provide insight as to factors contributing to differences in feed efficiency that may be exploited to improve feed efficiency in beef cattle herds.

Keywords Beef cattle · Rumen fluid · Metabolome

1 Introduction

The United States is both the largest producer and consumer of beef cattle globally (ERS 2019). In the beef cattle industry, approximately 40–60% of the total input cost comes from feed (Montaño-Bermudez et al. 1990). Identifying and incorporating methods for improving feed efficiency would not only potentially result in increased global protein availability, but would also result in decreased cost to producers and consumers. In order to select for more feed-efficient animals, these animals must first be identified, preferably through rapid, inexpensive methods. Untargeted metabolomics may provide a method for quickly and readily identifying more feed-efficient animals.

Untargeted metabolomics analyses aim to detect, quantify and subsequently identify as many metabolites within a given sample as possible (Fontanesi 2016). These metabolites can be both of endogenous and xenobiotic origin, with many of the xenobiotic metabolites derived from plants or microbiota (Fontanesi 2016). The metabolites measured are, by definition, intermediates or products of metabolism. Of particular interest with regards to feed efficiency are those metabolic processes involved in carbohydrate, fat, and protein metabolism (Nafikov and Beitz 2007). Alterations in intermediary metabolism partially contribute to divergences in feed efficiency (Ferrell and Jenkins 1984). Variations in those metabolites involved in intermediary metabolism could be suggestive of more or less efficient nutrient utilization, leading to more or less feed-efficient animals, respectively.

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Untargeted metabolomics, using ultrahigh-pressure liquid chromatography (UHPLC-MS), has been applied to serum from beef cattle, and differences in metabolite abundances were observed in steers varying in feed efficiency, as measured by differences in residual feed intake (RFI) (Clemmons et al. 2017). Clemmons and others found that some of the metabolites that differed in steers divergent in feed efficiency were those vital to intermediary metabolism, such as pantothenate and carnitine (Clemmons et al. 2017). The observed differences suggested variances in host metabolism, as serum metabolites are predictive of feed efficiency phenotypes in cattle (Clemmons et al. 2019b). Further studies performing untargeted and targeted metabolomic techniques have also found differences in rumen fluid and blood metabolites in cattle differing in feed efficiency (Artegoitia et al. 2017; Novais et al. 2019). However, the relationship between blood and rumen fluid metabolomes is not well-defined, and the blood metabolome may not be reflective of rumen function. Differences in the rumen fluid metabolome that are not reflected in the blood may still contribute to variation in feed efficiency.

Although untargeted metabolomics can provide a wealth of information, few studies have applied these techniques to examine the relationship between feed efficiency in beef cattle and the rumen fluid metabolome. The objective of this study was to use untargeted metabolomic techniques to identify metabolites in rumen fluid of beef steers that are associated with variation in feed efficiency phenotypes in beef steers.

2 Methods

2.1 Animal handling and sample collection

This study was approved and carried out in accordance with the recommendations of the University of Tennessee, Knoxville Institutional Animal Care and Use Committee.

The study utilized fifty purebred Angus steers from the University of Tennessee Institute of Agriculture Plateau Research and Education Center in Crossville, TN. Steers were 7 months of age and weighed 264 ± 2.7 kg at the start of the trial (Clemmons et al. 2017). Two weeks post-weaning, steers were transitioned for 10 days to a corn silage-based growing ration (11.57% crude protein and 76.93% total digestible nutrients on a dry matter basis) with 28 mg monensin/kg using the GrowSafe System© (GrowSafe Systems Ltd. Airdrie, Canada), which continually monitors feed intake, before beginning a 70 days feed efficiency trial. The steers remained on the diet and in the GrowSafe System© for the duration of the trial. Weekly, body weights were measured using a chute scale following the adaptation period for the duration of the 70 days feed efficiency trial. Feed intake was continually monitored following the adaptation period for 70 days. Residual feed intake was calculated as the difference between actual and expected dry matter intake (Koch et al. 1963). Following the end of the trial, steers were ranked based on RFI, and high- (n = 15) and low-(n = 14) RFI was chosen as those with 0.5 standard deviations above and below the mean, respectively. Rumen fluid was collected on the final day of the ten-week study via esophageal tubing and placed on ice for approximately two hours prior to processing. The animal handling and sample collection are further described by Clemmons et al. (2017).

For metabolomics analysis, a 2 mL aliquot of rumen fluid was transferred to sterile 2 mL microcentrifuge tubes and centrifuged for 15 min at 6000x*g* at 4 °C. The supernatant was aspirated using a 3 mL syringe with a 25G needle and filtered through a 0.22 µm pyrogen-free syringe filter (MidSci, St. Louis, MO, USA) into a 2 mL microvial. Filtered samples were stored at − 80 °C until further processing. The rumen fluid metabolome was extracted and analyzed similarly to Clemmons et. al. (Clemmons et al. 2017). Briefly, 50 µL of rumen fluid were extracted using 0.1% formic acid in acetonitrile:water:methanol (2:2:1) as previously described (Rabinowitz and Kimball 2007). A Synergy Hydro-RP column (100 × 2 mm, 2.5 µm particle size) was used to separate metabolites, with 10 µL of sample injected into the Dionex UltiMate 3000 UPLC system (Thermo Fisher Scientific, Waltham, MA). An Exactive™ (Rabinowitz and Kimball 2007) Plus Orbitrap MS (Thermo Fisher Scientific, Waltham, MA) was used to introduce samples via electrospray ionization, using an established method (Kamphorst et al. 2011; Lu et al. 2010).

2.2 Data analysis

The RAW files generated by Xcalibur MS software (Thermo Electron Corp. Waltham, MA) were converted to the.mzML format using the tool MS convert, a part of the suite of utilities in ProteoWizard software (Chambers et al. 2012). MAVEN (Metabolomic Analysis and Visualization Engine for LC–MS Data), a software package (Clasquin et al. 2012), that automatically performs non-linear retention time correction and calculates peak areas across samples, was used to pick known peaks. In MAVEN, a preliminary mass error of ± 10 ppm and retention time window of 5 min were used to detect peak areas across samples. Metabolites were annotated using expanded methods by Rabinowitz and others (Lu et al. 2010) based on retention time-accurate m/z pairs taken from MS1 spectra. The annotation parameters have been verified previously with pure standards as part of establishing the method. For a metabolite to be annotated as a known compound, the eluted peak had to be found within 2 min of the expected retention time, and the metabolite mass had to be within ± 5 ppm of the expected value. Data values

obtained from MAVEN were pre-processed and analyzed using Metaboanalyst 4.0 (Chong et al. 2018). Metabolites were median-centered, log-transformed, and pareto-scaled using Metaboanalyst 4.0. Metabolites differing significantly ($P < 0.05$) between low- and high-RFI steers were identified using student's *t*-test. Multiple testing was addressed by setting a false discovery rate of 5%, using the method of Benjamini and Hochberg (1995). Orthogonal partial least squares discriminant analysis (O-PLS-DA) was used to visualize separation between the two groups of steers based on serum metabolite profiles. The metrics R^2Y and Q^2 were used to evaluate the fit and prediction power, respectively, of the O-PLSDA model. Validity of model estimates was evaluated using permutation testing (Westerhuis et al. 2008). Sample labels were randomly assigned 1000 times, and new models were fitted and estimates of R^2Y and Q^2 were calculated for each random permutation. Values of R^2Y and Q^2 from the original model were compared to the distribution of values from the permutations to calculate empirical p-values for R^2Y and Q^2 (Online Resource 1). A principal component analysis was also used to visualize low- and high-RFI metabolomes and a variable importance in projection (VIP) plot with the top 25 variables. Pathway analyses to determine significant pathways impacted by RFI were analyzed in Metaboanalyst 4.0 using a global test with relative-betweenness centrality topology analysis with a reference library of *Escherichia coli* K-12 MG1655 (Kanehisa et al. 2013). Normality was assessed using the PROC UNIVARIATE procedure on raw, untransformed data in SAS 9.4 (SAS Institute, Cary, NC, USA). Normal distribution was considered based on a Shapiro–Wilk score of ≥ 0.85 and visual analysis of histogram and plots of residuals. Normally distributed metabolites were analyzed using one-way ANOVA in SAS with Tukey's HSD post-hoc test, whereas non-normally distributed variables were analyzed using Wilcoxon Rank Sum and Kruskal Wallis H test. Significance was set using $\alpha \leq 0.05$.

3 Results

A total of 88 known metabolites were identified. Eight metabolite abundances differed between low- and high-RFI steers based on either one-way ANOVA or Kruskal Wallis H test (Table 1). Orthogonal PLS-DA was used to visualize the discrimination between low- and high-RFI steers among rumen fluid samples (Fig. 1). The O-PLS-DA illustrated moderate overlap or similarity among animals differing in feed efficiency; however, two distinct metabolomes were present based on RFI (Fig. 1). A heatmap was

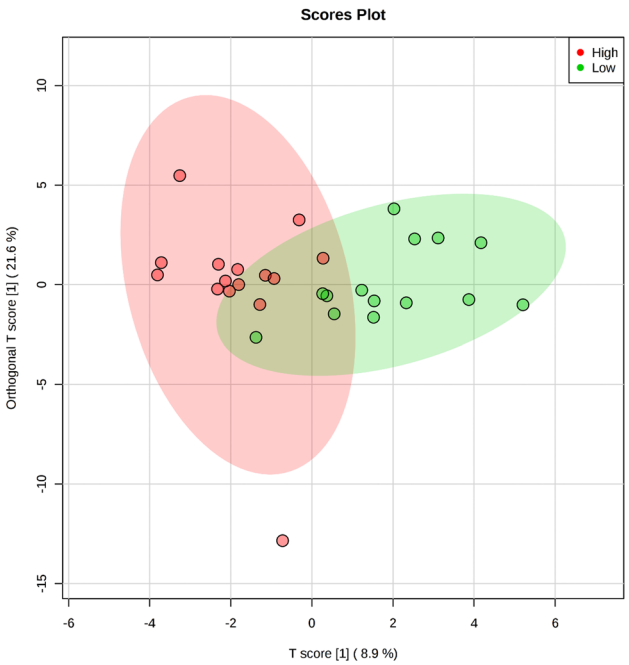

Fig. 1 Orthogonal partial least squares discriminant analysis of rumen fluid metabolites differing between high- and low- RFI steers. Ellipse represents 95% confidence interval

Table 1 Significant metabolites differing between high- and low-RFI

Metabolite	High-RFI[c]	Low-RFI[c]	P value[a]
3_4-dihydroxyphenylacetate (DOPAC)*	$1.96\times107\pm1.18\times106$	$3.85\times107\pm2.58\times106$	0.04
4-pyridoxate	$4.79\times108\pm5.93\times107$	$6.71\times108\pm4.62\times107$	0.02
Citraconate[b]	$1.53\times109\pm3.48\times108$	$8.42\times10^8\pm4.23\times10^8$	0.05
Hypoxanthine	$1.22\times109\pm1.21\times108$	$1.63\times109\pm1.09\times108$	0.02
Succinate/Methylmalonate	$3.44\times109\pm5.82\times108$	$6.35\times109\pm8.25\times108$	0.01
Thymine	$4.68\times108\pm6.15\times107$	$8.41\times108\pm1.39\times108$	0.02
Uracil	$1.04\times10^9\pm1.14\times10^8$	$1.51\times109\pm1.51\times108$	0.02
Xylose	$1.40\times108\pm1.42\times107$	$1.95\times108\pm1.67\times107$	0.02

[a]Significance determined at $P \leq 0.05$

[b]Analyzed using Kruskal Wallis *H* test

[c]Values are measured as mean ± SEM of area under the peak

also used for visualization of distribution of metabolites between high- and low-RFI steers (Fig. 2). The heatmap displays differences in the abundance of the top 25 metabolites of individual steers, with clustering by RFI (Fig. 2). Metabolite abundances by low- and high-RFI groups are presented in Online Resource 2. A PCA visualizing the low- and high-RFI rumen fluid metabolomes are also presented (Fig. 3). The VIP scores are presented based on the top 25 variables of importance are presented in Fig. 4. Several metabolic pathways were impacted as a result of RFI (Table 2).

4 Discussion

Feed efficiency is an important factor in the beef industry as consumers and producers realize the environmental and economic cost of producing animal proteins (Myer et al. 2019). The ruminal microbiota contribute significantly to feed efficiency and animal production by converting feed into energy and nutrients for the host animal, which are subsequently released in the rumen and available for absorption in both the rumen and lower gastrointestinal tract. The metabolites produced and released by the rumen microbiome have the potential to influence host phenotypes, and thus are

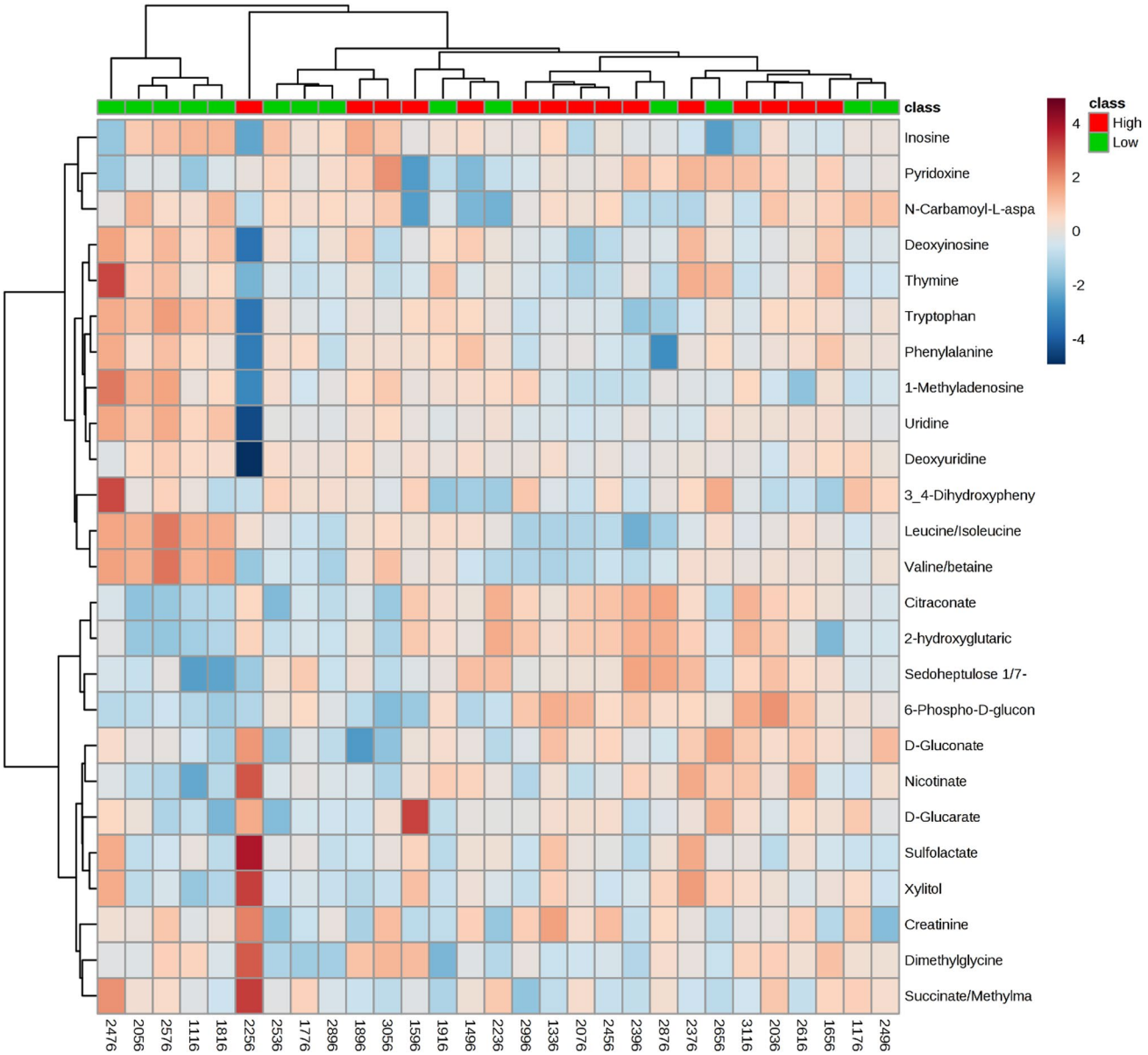

Fig. 2 Heatmap of the top 25 metabolites differing between high- and low-RFI steers

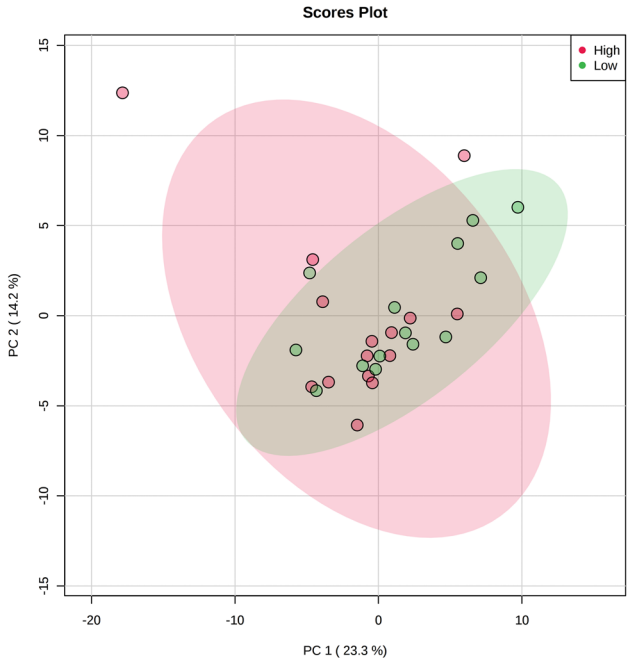

Fig. 3 Principal component analysis of rumen metabolomes of high- and low-RFI steers. Ellipse represents 95% confidence interval

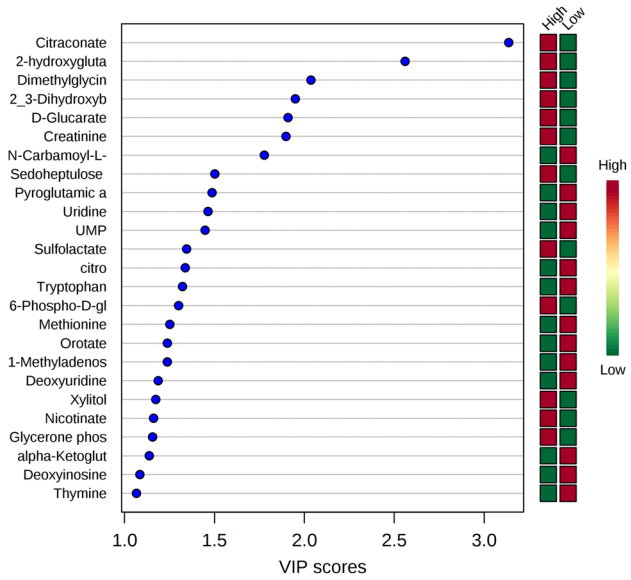

Fig. 4 Variable importance analysis of top 25 metabolites between high- and low-RFI steers

of particular interest for understanding contributing factors to variation in feed efficiency phenotypes.

Succinate/methylmalonate was greater in the rumen fluid of low-RFI compared to high-RFI animals in this study. Succinate is a precursor of propionate, whose primary fate is towards gluconeogenesis in ruminants (Van Gylswyk 1995; Yost et al. 1977). In the rumen, bacteria such as *Fibrobacter succinogenes* produce succinate, which is then converted to propionate by bacteria such as *Selenomas ruminantium* (Ushida et al. 1985; Weimer 1998). Succinate is converted to propionate through a multi-enzymatic process with the assistance of coenzyme A (CoA), in which CoA becomes bound to succinate, isomerized to methylmalonyl-CoA, converted to proponyl-CoA, which eventually produces propionate (Wirth et al. 2018). Propionate can then be absorbed through the rumen epithelium and used in the liver for gluconeogenesis. The greater abundance of succinate in the low-RFI (more efficient) steers may indicate more glucogenic precursors are available to the host through a rumen microbiome that may produce these glucogenic precursors more efficiently or effectively. Indeed, in a study conducted by Myer and colleagues, more efficient steers had greater abundances of succinate- and propionate-producing bacteria in the rumen, including *Succiniclasticum spp.* (Myer et al. 2015). Additionally, a study conducted by Clemmons et. al. found that steers with low-RFI had greater abundances of serum pantothenate, a precursor of CoA (Clemmons et al. 2017), which was found to be predictive of feed efficiency in steers (Clemmons et al. 2019a). This could potentially suggest that not only are the precursors available with the greater abundance of succinate/methylmalonate in this study, but more efficient steers could be utilizing these energy precursors more effectively since CoA is used in the conversion of succinate to propionate.

Table 2 Metabolic pathway analysis of pathways that differed significantly by low- and high-RFI steers

Metabolic pathway	Impact	P Value[a]
C_5-Branched dibasic acid metabolism	0.50	0.01
Valine, leucine and isoleucine biosynthesis	0.11	0.01
Ascorbate and aldarate metabolism	0.01	0.05

[a]Significance determined at $P \leq 0.05$

Succinate is also an intermediate of γ-aminobutyric acid (GABA) metabolism. Succinate is converted from succinate semialdehyde to succinate by succinate semialdehyde dehydrogenase (Blasi et al. 2002), and the succinate can then be converted back to GABA via the citric acid cycle as conversion to 2-oxoglutarate (Berg et al. 2002). The 2-oxoglutarate can then be converted back to GABA (Berg et al. 2002). The metabolite GABA is a potent neurotransmitter. In cattle, GABA is a significant mediator of feed intake and weight gain (Fan et al. 2015). Fan and colleagues (2010) performed a genome-wide association study (GWAS) across 807 Simmental cattle to determine single-nucleotide polymorphisms (SNP) associated with production-relevant traits related to feed intake and weight gain (Fan et al. 2015). The authors found that the GABAergic pathways were associated with both feed intake and weight gain according to nominal

P-values (Fan et al. 2015). The authors further describe the effect of providing exogenous GABA in other species, including the observed feed intake and weight gain in pigs (Fan et al. 2007) and dairy cattle (Wang et al. 2013). The greater abundance of succinate in this study could be a possible indication of greater GABA metabolism in cattle from any succinate that may have escaped ruminal fermentation, leading to differences in weight gain. Although further analyses are warranted to confirm these speculations, succinate could be a promising biomarker of feed efficiency from the rumen of beef cattle.

Several nucleic acids or metabolites associated with nucleic acid metabolism differed between low- and high-RFI groups. Uracil, thymine, and hypoxanthine were all greater in low-RFI steers compared to high-RFI steers. Hypoxanthine is a purine derivative, while uracil and thymine are nucleic acid bases found in RNA and DNA, respectively. The greater abundance of these nucleic acids may be indicative of higher turnover of microbial or plant genetic materials. The amount of purine metabolism derivatives found in urine are positively correlated with the amount of microbial nucleic acids that enter the small intestine (McAllan et al. 1982; Thanh and Ørskov 2006), and greater amounts of nucleic acids in the rumen can be indicative of greater availability of microbial crude protein (MCP) (Leng and Nolan 1984). Studies conducted by McAllan and Smith analyzed degradation of DNA and RNA in rumen fluid in vitro (McAllan and Smith 1973). They found that, while many components of DNA and RNA are completely or mostly degraded, hypoxanthine, uracil, and thymine resisted degradation after four hours of incubation (McAllan and Smith 1973). Of these three nucleic acids or nucleic acid derivatives, uracil was most resistant to degradation, with approximately 60% of the original uracil still present after the four hour incubation, followed by hypoxanthine (45% remaining), and thymine (30% remaining) (McAllan and Smith 1973). Given that these nucleic acids or derivatives are most resistant to degradation by the rumen microbiota, they may be indicators of genetic material turnover in the rumen. Because these were also found in greater abundances in the rumen of more feed-efficient steers, this may be indicative of more rapid turnover or increased production of microbial crude protein, especially given that these animals were on the same diets.

Pyrodixate, or pyridoxic acid, was also greater in low-RFI animals than in high-RFI animals. Pyridoxic acid is a byproduct of vitamin B_6 catabolism, and provides an estimate of the vitamin B_6 oxidation that occurs in humans (Linkswiler and Reynolds 1950). Vitamin B_6 is required for many biological reactions, including those involved in amino acid, carbohydrate, and fatty acid metabolism (Leklem and Machlin 1991; McCormick 2006; Merrill and Burnham 1990). Except in production stages requiring extensive nutrient requirements, such as early lactation in dairy cattle, the rumen microbiota and diet provide sufficient B vitamins to meet the need of the animal (Bechdel et al. 1928). Although B vitamins, including vitamin B_6, have demonstrated vital and wide-ranging functions in metabolism, there is little understanding or information about the use of vitamin B_6 supplementation to improve cattle production (Weiss and Ferreira 2006). Given that a vitamin B_6 metabolism byproduct was greater in the rumen of more feed-efficient animals, it is possible that vitamin B_6 metabolism is greater in the rumen of cattle. This may permit greater protein and muscle accretion, leading to a more efficient animal. Additionally, 4-pyridoxiate can be converted downstream to succinate semialdehyde by members of Proteobacteria (Blasi et al. 2002), one of the most abundant phyla in beef cattle rumen. Succinate semialdehyde can be irreversibly converted to succinate (Chambliss and Gibson 1992; Ryzlak and Pietruszko 1988), which was found to also be greater in the rumen of low-RFI beef cattle in this study. This could provide further support of the impact of vitamin B_6 turnover. However, this is speculative, and more research would be needed to support this concept.

Although other metabolites differed between low- and high-RFI steers, including citraconate (Krishnamachar et al. 1964), DOPAC (Gnegy 2012), and xylose (Pearson and Baldwin 1981), their biological roles are not well-known or understood. Citraconate is an intermediate of citric acid metabolism and xylose is a plant monosaccharide, which both may have contributed to carbohydrate metabolism, whereas DOPAC is a byproduct of dopamine metabolism (Gnegy 2012). Indeed, the C_5-branched dibasic acid metabolism was impacted as a result of high- and low-RFI, a pathway in which citraconate is involved (Kanehisa et al. 2013). Although the role of these three metabolites is not well-defined currently, they represent potential biomarkers of feed efficiency in beef cattle, or may represent future metabolites of interest to study in relation to feed efficiency in cattle.

The steers in this study were fed monensin, an ionophore that alters the rumen microbiome and is routinely used in the United States to improve feed efficiency and reduce bloat (Goodrich et al. 1984). Monensin has a demonstrated effect on the rumen microbial populations, microbial function, and the rumen fluid metabolome (Ogunade and Schweickart 2018; Ogunade et al. 2018). Although monensin alters both feed efficiency and the microbial populations and metabolism, all steers in this study received monensin and RFI separation was still observed. The rumen fluid metabolomes also differed as a result of RFI differences. The differences in rumen fluid metabolomes could be the result of several different factors, such as possible variation in the rumen microbiome and microbial metabolism as well as differences in host metabolism. Steers that differ in RFI and other measurements of feed efficiency have a demonstrated variation

in the rumen microbiome (Myer et al. 2015). Additionally, microbial differences by steers differing in feed efficiency were observed in animals also consuming monensin (Myer et al. 2015). Thus, potential differences in the rumen microbiome as a result of feed efficiency could alter the rumen metabolome. However, further studies analyzing the rumen metabolome in cattle differing in RFI not consuming monensin should be conducted in order to determine biomarkers of feed efficiency when cattle do not have access to the ionophore.

The differences in the rumen fluid metabolites as a result of feed efficiency could also be due to variation in host metabolism. Feed efficiency is a moderately heritable trait, suggesting that divergences in feed efficiency in beef cattle are the result of some host physiology (Arthur et al. 2001). Indeed, Kong et. al. analyzed the transcriptome of the rumen epithelium in high- and low-RFI steers using RNA-Seq (Kong et al. 2016). Kong and colleagues found that 122 genes were differentially expressed between high- and low-RFI steers, with increased expression of genes related to energy utilization via glycolysis and the tricarboxylic acid cycle, as well as an increased number of mitochondrial gene copy number (Kong et al. 2016). This potentially relates to some of the metabolites observed in this study that are intermediates of these metabolic processes. Citraconate and succinate are both intermediates of the tricarboxylic acid cycle, and were in greater abundance in the rumen of low-RFI cattle. Additionally, Kong and colleagues demonstrated that there was enrichment of genes regulating protein turnover (Kong et al. 2016). The greater abundance of nucleic acids observed in the rumen fluid low-RFI steers in the present study may be related to this, as greater protein and/or genetic material turnover could result in greater abundances of nucleic acids being released into the rumen. Further studies utilizing both transcriptomics and metabolomics or other pathway analyses could corroborate this hypothesis.

Although several metabolites differed in steers divergent in feed efficiency, the heatmap illustrated some overlap of steers from both phenotypes. There may be several possible reasons for this. First, all steers were on the same diet and maintained under the same conditions. Given this, it stands to reason that the byproducts of catabolism of the feedstuffs would be similar across all animals, regardless of phenotype. Eight of the 88 known metabolites observed in this study differed between low- and high-RFI steers, thus the remaining 80 metabolites could have contributed to the overlap of feed efficiency phenotypes observed in the heatmap. Additionally, one of the greatest sources of variation in ruminant microbiomes or rumen environment besides diet is individual animal variation (Henderson et al. 2015). The rumen metabolome as a whole may not be highly correlated or indicative of feed efficiency given that the host and its diet contribute to the rumen fluid metabolome. Although the study was conducted for a duration long enough to observe stabilization of the rumen microbiome (Clemmons et al. 2019b), as well as enough experimental units to observe differences between low- and high-RFI, having additional animals or conducting the study for a longer length of time may have allowed us to discern greater separation in the rumen metabolomes of cattle differing in feed efficiency.

In this study, several metabolites differed in the rumen fluid of steers differing in feed efficiency. Most of these metabolites are involved in genetic material recycling and protein metabolism, in addition to others that are involved in carbohydrate and lipid metabolism. Not only could the identified metabolites be potential biomarkers of feed efficiency, but they could also provide insight into the metabolic, microbial, and physical differences that result in variation of feed efficiency in cattle. In the future, additional studies should be conducted to expand upon these results, such as analyzing the rumen fluid metabolome in female cattle at different production stages. Additionally, the roles of the individual metabolites identified in this study could be examined in the greater context of feed efficiency in cattle, as well as investigating the relationship among beef cattle feed efficiency, the rumen microbiome, and the rumen fluid and serum metabolomes to gain a comprehensive insight into the impacts of microbial rumen function on host metabolism and feed efficiency.

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Author contribution BAC performed research, analyzed data, and wrote the paper. JBP contributed analytical tools and analyzed data. SRC contributed analytical tools and analyzed data. TBS analyzed data and wrote segments of the paper. MME conceived of or designed study, performed research, and analyzed data. PRM conceived of or designed study, performed research, analyzed data, and wrote the paper.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest BA Clemmons, JB Powers, SR Campagna, TB Seay, and PR Myer declare that they have no conflict of interest. MM Embree is the Co-Founder and Chief Science Officer at Ascus Biosciences, Inc.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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