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Exploring the rumen microbial function in Angus bulls with divergent residual feed intake

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This study leverages Shotgun metagenomics to assess the rumen microbial community and functionality in Angus bulls with differing residual feed intake-expected progeny difference (RFI-EPD) values, aiming to elucidate the microbial contributions to feed efficiency. Negative RFI-EPD bulls (NegRFI: n=10; RFI-EPD= -0.3883 kg/d) and positive RFI-EPD bulls (PosRFI: n=10; RFI-EPD=0.2935 kg/d) were selected from a group of 59 Angus bulls (average body weight (BW) = 428 ± 18.8 kg; 350 ± 13.4 d of age) fed a high-forage total mixed ration after a 60-d testing period. At the end of the 60-d period, rumen fluid samples were collected for bacterial DNA extraction and subsequent shotgun metagenomic sequencing. Results of the metagenome analysis revealed greater gene richness in NegRFI bulls, compared to PosRFI. Analysis of similarity revealed a small but noticeable difference (P =0.052; R-value = 0.097) in the rumen microbial community of NegRFI and PosRFI bulls. Linear Discriminant Analysis effect size (Lefse) was utilized to identify the differentially abundant taxa. The Lefse results showed that class *Fibrobacteria* (LDA = 5.1) and genus *Fibrobacter* (LDA = 4.8) were greater in NegRFI bulls, compared to PosRFI bulls. Relative abundance of the carbohydrate-active enzymes was also compared using Lefse. The results showed greater relative abundance of glycoside hydrolases and carbohydrate-binding modules such as *GH5, CBM86, CBM35, GH43*, and *CBM6* (LDA > 3.0) in NegRFI bulls whereas *GH13* and *GT2* were greater in PosRFI bulls. The distinct metabolic and microbial profiles observed in NegRFI, compared to PosRFI bulls, characterized by greater gene richness and specific taxa such as *Fibrobacter*, and variations in carbohydrate-active enzymes, underscore the potential genetic and functional differences in their rumen microbiome. These findings contribute to a deeper understanding of the interplay between rumen microbiota and feed efficiency in Angus bulls, opening avenues for targeted interventions and advancements in livestock management practices.

KEYWORDS

ruminants, metagenomics, microbiome, feed efficiency, beef cattle and bull

Introduction

In the cattle industry, feed costs make up the largest part of production expenses causing efficient bull breeding is becoming increasingly more important (Wang et al., 2012). Even though feed costs are the main factor in profitability, genetic selection programs have usually focused on increasing weight gain rather than reducing feed intake (Alende et al., 2016). Furthermore, the beef cattle industry is facing more scrutiny due to environmental and economic concerns, leading to a push for more sustainable practices.

Residual feed intake (RFI), defined as the difference between actual and expected dry matter intake, is moderately heritable (h2 ≈ 0.35). This indicates that selecting for efficient or negative-RFI cattle will result in progeny that consume less feed compared to their less efficient or positive-RFI counterparts (Kenny et al., 2018; Arthur and Herd, 2008). Additionally, RFI is associated with various cattle traits such as mitochondrial efficiency and growth patterns (Idowu et al., 2022; Taiwo et al., 2022b). Expected Progeny Differences (EPDs) predict the genetic potential of offspring, and recent efforts have incorporated RFI into EPDs to select for more feed-efficient cattle. This integration removes environmental influences, allowing for comparisons within breeds across environments (Rossi et al., 2022; Beck, 2022). Research in this area offers opportunities for optimizing progeny without affecting bull reproductive parameters (Rossi et al., 2022).

While diet has long been considered the primary determinant of gut microbiota composition, recent research highlights the heritability of the cattle microbiome as a significant factor (Gonzalez-Recio et al., 2018). Ruminal fermentation is crucial for energy provision from feed, emphasizing the importance of feed efficiency (Guan et al., 2008). Progress in microbiome research has previously underscored the genetic influence on ruminal microbial composition and function. This research may further identify microbial signatures associated with feed efficiency, aiding targeted breeding and microbial selection programs to enhance overall beef cattle productivity (Li et al., 2019a).

Metagenomics is a powerful tool for unraveling microbial communities and their functional potential in diverse ecosystems (Handelsman, 2004; Yung et al., 2009). While next-generation sequencing has revolutionized genomics, choosing between Shotgun metagenomics sequencing and 16S rRNA gene sequencing is crucial. Although 16S rRNA gene sequencing provides taxonomic information, it is limited in resolving biological functions (Pace et al., 1986; Sharpton, 2014). Shotgun metagenomics sequencing offers a broader view of microbial diversity and functional capabilities, making it a preferred method for in-depth microbial studies (Basbas et al., 2023; Robinson et al., 2021). The integration of shotgun metagenomics into ruminant science has been reported to yield a more precise evaluation of microbial diversity and functional potential, as well as their impact on feed efficiency (Delgado et al., 2019; Xie et al., 2022). Thus, the objective of this study was to leverage deep Shotgun metagenomics sequencing to assess differences in the rumen microbial community and function in Angus bulls with negative or positive RFI-EPD.

Materials and methods

Animals, diet, RFI-EPD determination, and sampling

The research procedures were approved by the West Virginia University's Institutional Animal Care and Use Committee (IACUC Protocol Number: 2206054350). A group of 59 Angus bulls (average body weight (BW) = 428 ± 18.8 kg; 350 ± 13.4 d of age) were fed a high-forage total mixed ration (TMR; primarily consisting of corn silage, hay, cracked corn, and a ration-balancing supplement; see Table 1) for 60 days in two pens (pen 1: n = 31; pen 2: n = 28). Each pen was equipped with two GrowSafe8000 intake nodes (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) to measure individual feed intake and In-Pen Weighing Positions (IPW, Vytelle LLC), positioned at a water trough in each pen to measure the body weight (BW) of individual animals several times daily (Wells et al., 2021). The use of IPW to measure BW has

TABLE 1 Ingredient and chemical composition of the basal diet Ingredients (%DM).

	% of dietary DM
Corn silage	65.61
Hay[a]	14.93
Cracked corn	14.50
Concentrate supplement[b]	4.69
Mineral/vitamin mix[c]	0.27
Nutrient Analysis	
DM %	48.3
Crude Protein %	11.6
NDF %	38.5
NFC %	42.0
Fat %	3.59
Calcium %	0.57
Phosphorus %	0.37
Potassium %	1.28
Magnesium %	0.15
NE_m, Mcal/kg	1.71
NE_g, Mcal/kg	1.13

[a]Contains a blend of Smooth Broom hay, Timothy hay, and Orchard Grass.
[b]50% concentrate Supplement (Kalmbach Feeds, Pennsylvania, PA) contained soybean meal, corn dried distillers grains (DDGS), soybean hulls, lime-calcium supplement, urea, mold star dry, salt, monocal (containing monofluorophosphate and calcium carbonate), magnesium oxide, K-Dairy Premix (containing vitamin A, vitamin D, vitamin, E, antioxidant, manganese, zinc, iron, copper, iodine, cobalt, magnesium, and selenium), Zinpro Availa 4 (containing zinc, manganese, copper, and cobalt), Rumensin 90 (containing monensin; 90.7 g/lb), selenium, vitamin A, Tylan 40 (containing tylosin phosphate; 40 g/lb), Alkosel (containing selenium enriched yeast; 3000 ppm Se), vitamin D3, vitamin E, Kem Trace chromium; guaranteed analysis: 44% crude protein; 2.6% crude fat; 9.7% crude fiber; 13.2% ADF; 18.9% NDF; 10.4% non-protein nitrogen.
[c]Contains calcium, phosphorus, magnesium, potassium, ash, sulfur, sodium, chloride, iron, manganese, zinc, copper, and molybdenum; guaranteed analysis (% DM): 5.77% ash; 0.57% Ca; 0.37% P; 0.15% Mg; 1.28% K, 0.16% Na.

enabled the measurement of feed efficiency with sufficient accuracy over a test period of 59 days (MacNeil et al., 2021; Wells et al., 2021; Taiwo et al., 2022a). Following this test period, growth performance and feed intake data were collected. The phenotypic RFI values of the bulls were determined as described previously by Taiwo et al., 2022a. Briefly, the daily BW was regressed on time to calculate the beginning BW, mid-test BW, and average daily gain (ADG). Thereafter, ADG and metabolic mid-test BW (mid-test BW0.75) were regressed against individual daily DMI, and RFI was calculated as the difference between the predicted value of the regression and the actual measured value using the following equation; $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon$, where Y is the dry matter intake (DMI; kg/d), β_0 is the regression intercept, β_1 and β_2 are partial regression coefficients, X_1 represents the metabolic mid-test BW (MMTW = mid test $BW^{0.75}$; kg), and X_2 is the average daily gain (ADG; kg/d) (Durunna et al., 2011; Taiwo et al., 2022a). Genetic evaluation of the bulls was performed by Vytelle (Vytelle Insight Beef Genetics) using data collected by Vytelle SENSE systems and Vytelle INSIGHT analytics services, which include at least three generations of pedigrees to determine the RFI-EPD values. At the conclusion of the trial and subsequent calculations, the bulls were ranked based on their Residual Feed Intake Expected Progeny Difference (RFI-EPD) coefficients. The bulls with the most negative RFI-EPD values (NegRFI; genetically efficient; n = 10) and the most positive RFI-EPD values (PosRFI; genetically inefficient; n = 10) were identified for further evaluation.

Rumen fluid samples were collected prior to morning feeding on day 60 of the testing period from all the bulls using an orally administered stomach tube connected to a vacuum pump (Ruminator; profs-products.com). The first 200 mL of rumen fluid was discarded to prevent saliva contamination. Then, 200 mL of fluid was collected from each animal and placed into 50-mL polypropylene conical bottom tubes. The samples were immediately placed on ice after collection and subsequently stored at −80°C until DNA extraction and sequencing were performed.

DNA extraction

Before DNA extraction, rumen fluid samples from Angus bulls identified as NegRFI and PosRFI were thawed at room temperature. Microbial DNA was extracted from 500 µL of rumen fluid samples using the Qiagen DNeasy Powersoil Pro DNA Isolation Kit following the manufacturer's instructions (Qiagen; catalog number: 47014, Germantown, MD, USA). Using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), total DNA purity was measured, with an A260/A280 ratio ranging from 1.8 to 2.0. To prevent contamination, Kimtech wipes (Fisher scientific; catalog number 06-666, Pittsburg, PA, USA) were utilized throughout spectrophotometry quantification steps prior to each sample measurement. DNA was stored at −80°C until sequencing.

Metagenomic sequencing, bioinformatic analysis, and statistical analysis

All subsequent steps were performed at Novogene Bioinformatics Technology (UC Davis Sequencing Center, CA; Batch ID: X202SC23094803-Z01-F001; Contract ID: H202SC23094803). For library preparation and construction, quality control (QC) of DNA samples was performed using agarose gel electrophoresis to assess DNA degradation and potential contamination. The concentration of DNA in the library was measured using a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) with a Qubit® dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The purity of samples ranged from 1.8 to 2.0, with concentrations greater than 1 µg used to construct the library. Following library construction, the DNA was measured using the Qubit Fluorometric Quantification system (Thermo Fisher Scientific, Waltham, MA, USA). Genomic DNA of each sample was randomly sheared into short fragments (350 bp) for sequencing and processed using the Illumina NovaSeq 6000 PE150bp sequencing platform according to its effective concentration and expected data volume, as described by Li et al. (2022).

Preprocessing of raw data from the Illumina sequencing platform was performed to obtain clean data for subsequent analysis. Clean data were obtained by removing low-quality bases (default quality ≥ 38) exceeding the default length of 40 bp, reads containing N bases exceeding the default length of 10 bp, and reads with overlaps with adapters exceeding the default length of 15 bp. Subsequently, clean data were BLASTed against the host database (*B. Taurus* ARS-UCD2.0)., using Bowtie2 software (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) by default, followed by MEGAHIT software for assembly analysis of clean data. For Scaftigs without N assembly, resulting Scaffolds from the N junction were broken as previously reported (Qin et al., 2010; Li et al., 2015).

DIAMOND software (https://github.com/bbuchfink/diamond/; Buchfink et al., 2015) was used to align unigenes to the sequences of bacteria, fungi, archaea, and viruses from the non-redundant National Center for Biotechnology Information (NCBI) database. To prevent multiple alignment results and ensure species annotation and abundance of the sequences, a Lossless Compression Algorithm (LCA) was applied in the taxonomic software MEGAN. To form leveled taxonomic abundance tables, a relative abundance overview, and an abundance clustering heatmap, principal coordinate analysis (PCA) was performed. To identify differences between groups, analysis of similarities (ANOSIM), Metastat, and linear discriminant analysis (LDA, default score of 4) effect size (LEfSe) were conducted.

To evaluate the rumen microbial function, DIAMOND software (https://github.com/bbuchfink/diamond/) was used to BLAST Unigenes alignment with the functional database according to the default parameter settings of BLAST. The functional databases include KEGG (http://www.kegg.jp/kegg/) and CAZy (http://www.cazy.org/). Additionally, LEfSe analyses

(LDA ≤ 2) of the functional differences between the two groups were conducted.

The growth performance data (DMI, average daily gain (ADG), initial and final BW) and RFI values of the PosRFI and NegRFI Angus bulls were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Animals were included as a random effect nested within RFI-EPD. The RFI-EPD status was included as a fixed effect, and initial body weight values were included as a covariate for the final body weight. Significant effects were reported at $P \leq 0.05$.

Results

Growth performance

The results of the growth performance of the NegRFI and PosRFI bulls are shown in Table 2. The average RFI-EPD values of the NegRFI and PosRFI bulls were -0.39 kg/d and 0.29 kg/d, respectively. The initial and final body weights and ADG were similar ($P > 0.05$) between the groups. However, the DMI was lower ($P = 0.0002$) in the NegRFI bulls (7.74 kg/d) compared to the PosRFI bulls (9.90 kg/d).

The rumen metagenome profile

A total of 132, 526, 840 raw reads were obtained from the 20 rumen fluid samples of the Angus bulls, with an average of 6,626,342.00 ± 815,282.6 raw reads per sample. After quality control and removal, 131,849, 910 clean reads, with 6,592,455 ± 803,592 reads per sample, were retained (Supplementary Table 1). Using Megahit, a total number of 6,993,174 scaftigs with an average of 349,659 ± 72,350 scaftigs per sample were obtained by interrupting scaffolds at the N-site, resulting in an average max length of 170,278bp (Supplementary Table 2).

TABLE 2 Growth performance of Angus bulls selected for divergent residual feed intake.

Item	PosRFI	NegRFI	SEM	P-value
RFI-EPD, kg/d	0.29	-0.39	0.05	<.0001
RFI, kg/d	2.09	-2.55	0.68	<.0001
Initial body weight, kg	350	325	19.60	0.22
Final body weight, kg[1]	427	429	7.33	0.82
Final body weight, kg[2]	440	417	20.23	0.28
ADG, kg/d	1.50	1.54	0.11	0.71
DMI, kg/d	9.90	7.74	0.15	0.0002

NegRFI, Angus bulls with negative residual feed intake; PosRFI, Angus bulls with positive residual feed intake; SEM, Standard error of means; RFI-EPD, Residual feed intake-expected progeny difference; DMI, Dry matter intake; ADG, Average daily gain.
[1]Covariate adjusted; [2]Non-covariate adjusted.

The rumen taxonomic profile

The results revealed numerically greater gene richness in NegRFI bulls compared to PosRFI bulls (Figure 1). ANOSIM results revealed a small but noticeable difference ($P = 0.052$; R = 0.097) between the rumen microbial communities of the two groups (Figure 2). However, the results of the Metastats analysis identified a total of 35 differentially abundant taxa at the species level. The relative abundance of 29 species such as *Fibrobacter* UWB5, UWT2, UWB1, *Trepomena* C6AB and *Trepomena* JC4 were greater in NegRFI bulls, whereas 6 species such as *Frischella japonica*, *Prevotella veroralis*, and *Helicobacter cetorum* were more abundant in PosRFI bulls (Supplementary Figure 1). Abundance heatmaps showing the distribution of the 35 dominant taxa at the phylum and species levels are reported in Supplementary Figures 2, 3. Figure 3 shows the principal coordinate analysis (PCA) plot based on the differentially abundant ($P < 0.05$) species. Results of the LEfSe analysis showed the class *Fibrobacteria* (LDA = 5.1) and genus *Fibrobacter* (LDA = 4.8) as the most differentially enriched taxa in NegRFI bulls compared to PosRFI bulls (Figure 4).

The rumen functional profile

Utilizing the first-level KEGG orthology database, 46 core pathways belonging to 6 functional categories were identified within the rumen microbiome of the bulls. Predominant genes involved in carbohydrate metabolism, amino acid metabolism, translation, replication and repair, and folding, sorting and degradation were identified as the top 5 most abundant functions (Figure 5). No differences in KEGG functional pathways were detected between the two groups.

To identify the relative abundance of carbohydrate-active enzymes (CAZy), predicted genes were annotated to the CAZy database containing the following 6 classes: glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), and auxiliary activities (AA). The relative distribution of all annotated CAZy genes is shown in Figure 6. The LEfSe analysis of CAZy genes revealed that the relative abundance of GH5, CBM86, CBM35, GH43, and CBM6 (LDA ≥ 2.0; $P \leq 0.05$) was greater in NegRFI bulls, whereas the relative abundance of GH13 and GT2 was greater in PosRFI bulls (LDA ≥ 2.0; $P \leq 0.05$) (Figure 7).

Discussion

Gene richness and performance traits

While the beef cattle production industry provides food and nutrition globally, feed efficiency is economically important for creating sustainable practices (de Ondarza and Tricarico, 2017; Stewart et al., 2018). Within ruminants, the influence of the rumen microbiome is crucial in facilitating feed intake, metabolism of carbohydrates, and energy utilization through the production of

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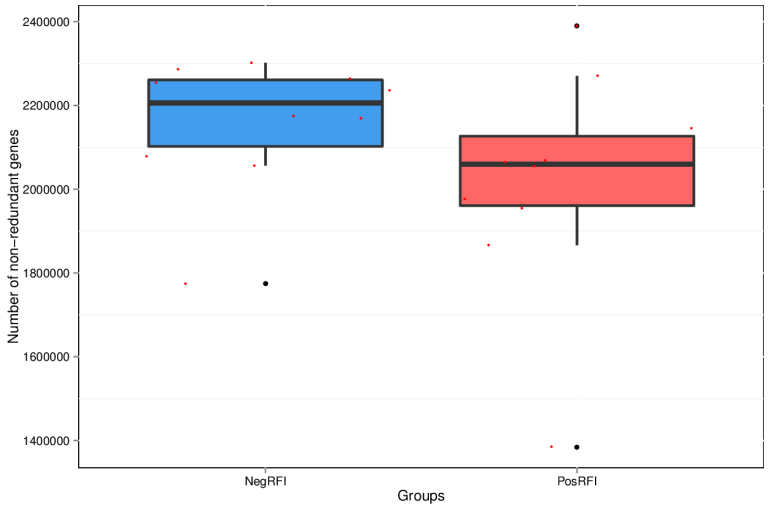

FIGURE 1
Total number of non-redundant genes in PosRFI and NegRFI bulls. X-axis coordinate stands for each group and the Y-axis coordinate stands for the number of non-redundant genes. NegRFI, Angus bulls with negative residual feed intake; PosRFI, Angus bulls with positive residual feed intake.

VFAs and nitrogen (Wang et al., 2013; Shabat et al., 2016; Wang et al., 2020). In this study, the quantitative gene abundance was greater in bulls identified as NegRFI. While little has been reported regarding a tendency of different gene richness in the rumen of cattle selected for feed efficiency, a study by Lima et al. (2019) reported a substantial link between rumen microbial abundances and feed intake, associating efficient cattle with greater gene abundance in the rumen while consuming less feed. Our research indicates an association between ruminal gene richness and genetic feed efficiency.

In cattle, the rumen serves as a station for diet fermentation, as it harbors a diverse microbial community that contributes to the breakdown of complex plant materials into absorbable nutrients, mediated by several mechanisms. These processes all contribute to cattle metabolic homeostasis (Sakata and Tamate, 1979; Lima et al., 2019). Relative to gene abundance, the expression of genes related to feed metabolism within the rumen microbiome significantly influences nutrient conversion and energy acquisition, ultimately impacting overall metabolic efficiency and production outcomes in cattle. The results of this study indicate greater gene richness in NegRFI bulls, which could suggest a greater ability to metabolize feed, requiring quantitatively less feed to exhibit the same production gain as their less efficient counterparts. However, conclusive results

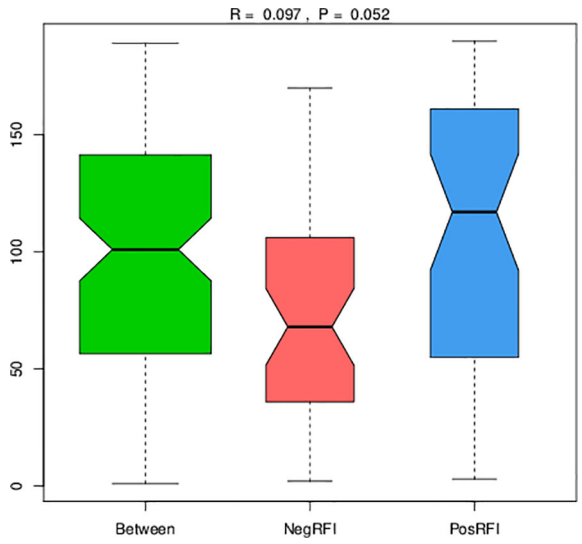

FIGURE 2
Analysis of similarity (ANOSIM) of the rumen microbiome of NegRFI and PosRFI Angus bulls. NegRFI, Angus bulls with negative residual feed intake; PosRFI, Angus bulls with positive residual feed intake.

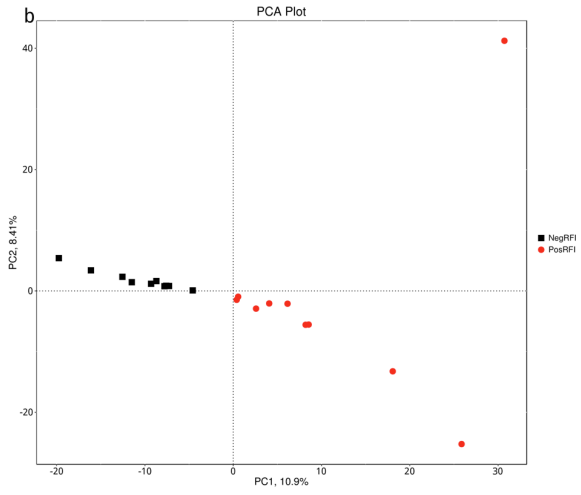

FIGURE 3
Principal component analysis (PCA) plot of the species whose abundance were significantly different between NegRFI and PosRFI ($P \leq 0.05$). NegRFI, Angus bulls with negative residual feed intake; PosRFI, Angus bulls with positive residual feed intake.

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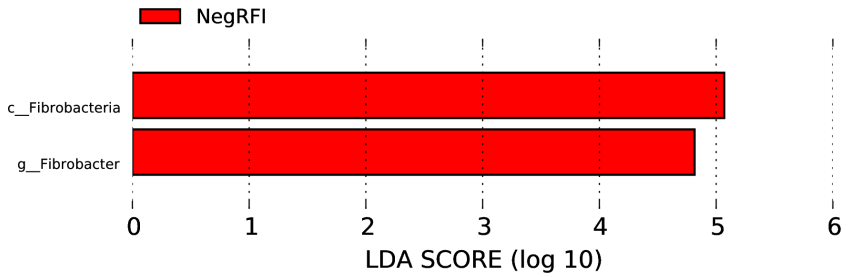

FIGURE 4
Least discriminant analysis effects size (LEfSe) analysis of the rumen microbiota of NegRFI and PosRFI Angus bulls. The linear discriminant analysis (LDA) plot indicates the most differentially abundant taxa by ranking according to their effect size (LDA ≥ 4). NegRFI, Angus bulls with negative residual feed intake; PosRFI, Angus bulls with positive residual feed intake.

surrounding microbial diversity and gene richness in feed-efficient cattle are far from finalized. In a study conducted by Shabat et al. (2016), cattle identified as feed efficient revealed a less diverse and genetically rich microbial community. Nevertheless, while these cattle were reported as such, they were accompanied by a significantly higher dominance of certain taxa involved in energy metabolism and VFA production at the genus and species level, which complements our results. While the results surrounding microbial community and gene richness are still widely variable, feed-efficient cattle consistently possess dominant taxa that contribute to energy metabolism and production (Myer et al., 2015; Shabat et al., 2016; Auffret et al., 2020).

Taxonomy and microbial community

Cattle possess a diverse microbial community in their rumen, characterized by dominant phylogenetic taxa such as Firmicutes, Fibrobacterota, Bacteroidetes, and Proteobacteria, which are crucial for efficient digestion and nutrient metabolism (Jami and Mizrahi, 2012; Myer et al., 2015). While a core microbiome exists in the rumen, research has exhibited altered rumen microbial profiles in cattle selected for feed efficiency (Li et al., 2019b; Idowu et al., 2023). In our study, the microbial communities between PosRFI and NegRFI bulls were different and can be explained by the greater gene abundance in NegRFI bulls. Additionally, the relative abundance of 35 species belonging to taxa such as *Fibrobacter*,

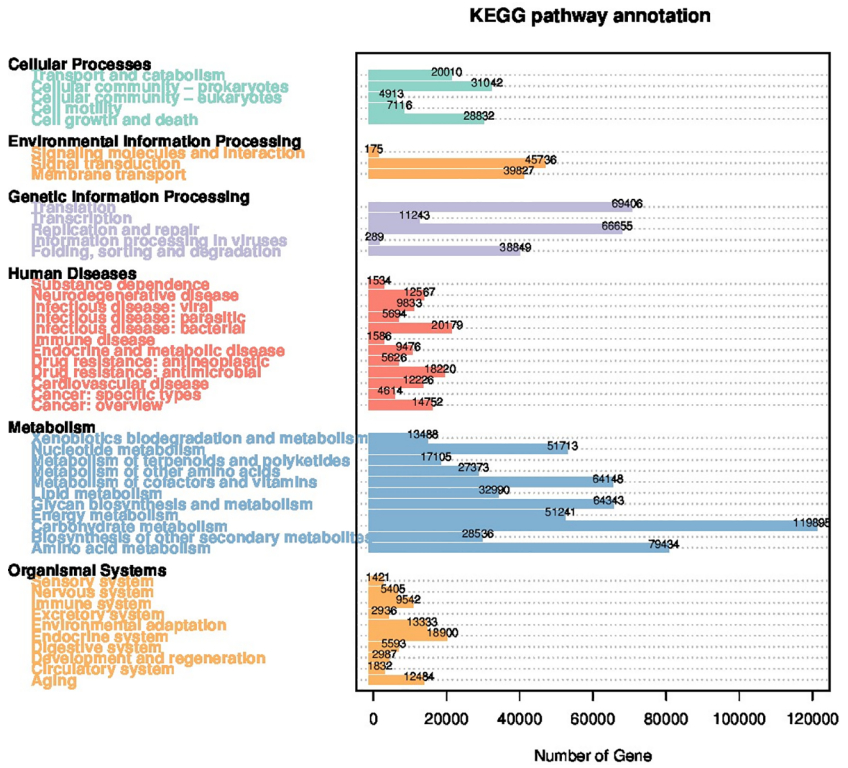

FIGURE 5
Distribution of category of the predicted genes by KEGG orthology annotation.

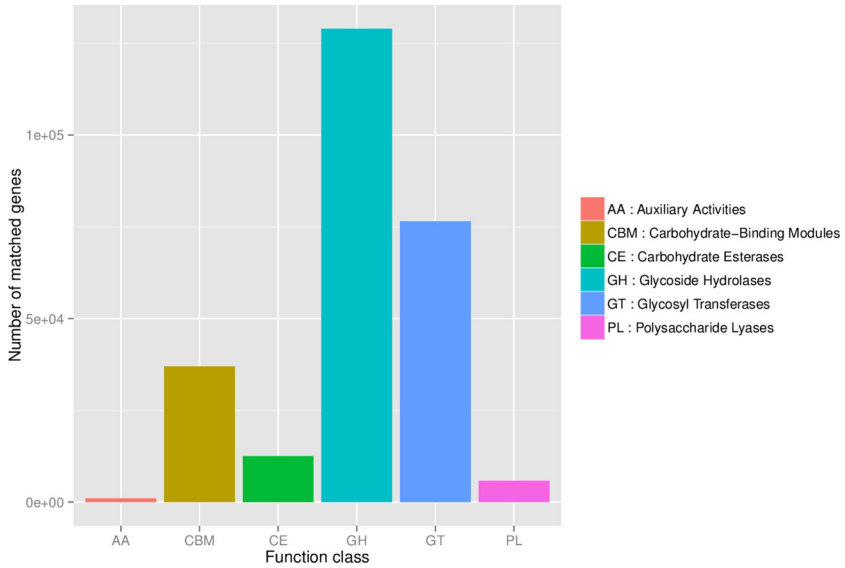

FIGURE 6
Relative abundance of the category of carbohydrate-active enzymes.

Lentisphaerae, *Bacteroidetes*, and *Treponema* were found to be different between the two groups of bulls.

The relative abundance of the genus *Treponema* and its species *T.* C6A8, *T.* JC4, and *T. porcinum* were greater in bulls identified as NegRFI, compared to PosRFI. Many *Treponema* species have been reported within the rumen of cattle and play a crucial role in the complex microbial ecosystem of the rumen; furthermore contributing to the efficient breakdown of fibrous plant materials and the production of energy-rich VFAs for the host animal (Radolf, 1996; Nordhoff et al., 2005; Rosewarne et al., 2012). The relative abundance of *Bacteroidetes* bacterium sp. Adurb.BinA104, *Candidatus Endolissoclinum*, and *Lentisphaerae* bacterium sp. GWF2528 were greater in NegRFI bulls compared to PosRFI bulls. These bacteria are classified as symbiotic proteobacteria and their phylogeny has previously been identified within the rumen of cattle divergent in feed efficiency (Kwan et al., 2012; Myer et al., 2015; Reis et al., 2023). Symbiotic proteobacteria contribute to ruminal fermentation through hydrolysis of complex polysaccharides, contributing to the formation and fermentation of biofilms, and have been previously reported to be involved in nucleotide, carbohydrate, and nitrogen metabolism (Hart et al., 2018; Zhang et al., 2021; Hernández et al., 2022). *Schwartzia* sp. *succinovorans* is a gram-negative bacterium that can ferment succinate to propionate in the rumen and was found in greater abundance in NegRFI bulls compared to PosRFI bulls (van Gylswyk et al., 1997). In ruminants, the production of VFAs is crucial in anaerobic digestion processes, serving as substrates for microbial metabolism (Bergman, 1990). Propionate and succinate use acetate: succinate transferases, which feed into the tricarboxylic acid (TCA) cycle, thereby potentially enhancing energy generation and microbial growth efficiency in the rumen (Kwong et al., 2017).

The relative abundance of species such as *Fibrobacter* UWB5, *F.* UWT2, and *F.* UWB were greater in NegRFI bulls compared to PosRFI bulls. Additionally, the genus *Fibrobacter* and the class

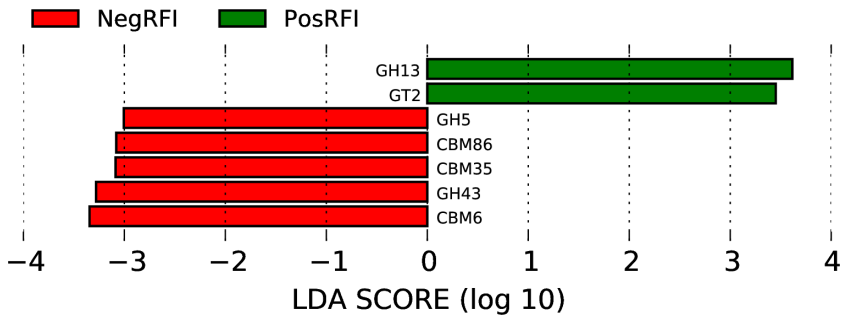

FIGURE 7
Least discriminant analysis effects size (LEfSe) plot showing the differentially abundant carbohydrate-active enzymes between PosRFI (green) and NegRFI (red) Angus bulls (LDA ≥ 2; $P \leq 0.05$). NegRFI, Angus bulls with negative residual feed intake; PosRFI, Angus bulls with positive residual feed intake; GH13, glycoside hydrolases family 13; GT2, glycoside transferases family 2; GH5, glycoside hydrolases family 5; CBM86, carbohydrate-binding modules family 86; CBM35, carbohydrate-binding modules family 35; CBM43, glycoside hydrolases family 43; and CBM6, carbohydrate-binding modules family 6.

Fibrobacteria were identified as the most enriched taxa in NegRFI Angus bulls. Fibrobacteria, particularly represented by the genus *Fibrobacter* and its species *F.* UWB5, *F.* UWT2, and *F.* UWB, are major cellulose-degrading bacteria. They break down plant material, including cellulose and hemicellulose, through the secretion of fibrolytic enzymes (Suen et al., 2011; Cammack et al., 2018). This enzymatic activity facilitates the breakdown of complex polysaccharides into simpler compounds, enhancing the accessibility of nutrients for both the microbial community and the host animal (Comtet-Marre et al., 2017; Jewell et al., 2013). A study by Neumann and Suen (2018) highlighted the importance of herbivore-associated *Fibrobacter* spp. within the gut microbiome of ruminant livestock, noting that species *F.* UWB5, *F.* UWT2, and *F.* UWB are associated with lignocellulose degradation.

Lignocellulose, an important building block in plant cell walls, is among the most abundant natural feedstocks in agriculture (Vázquez-Vuelvas et al., 2021). Cattle heavily rely on microbial digestion of plant lignocellulosic roughages, which make up major parts of their diets and consist mostly of lignin and three polysaccharides: cellulose, hemicellulose, and pectin (Naraian and Gautam, 2018; Gharechahi et al., 2023). The breakdown and conversion of complex plant material into polysaccharides within the rumen are suggested to support up to 70% of a ruminant animal's daily energy requirements (Flint et al., 2008). This suggests that NegRFI bulls are able to break down and utilize feed more efficiently for increased energy production and metabolism, which likely explains their better feed efficiency.

The increased abundance of fiber-degrading species in the rumen of NegRFI bulls suggests an enhanced capacity to break down and utilize fibrous components of high-forage diets, compared to PosRFI bulls. This improved fiber degradation likely supports the ability of these bulls to grow at similar rates while consuming less feed compared to NegRFI bulls. Improved fiber degradative species in cattle selected for feed efficiency have previously been reported (McGovern et al., 2018; Elolimy et al., 2018; Liu et al., 2022). McGovern et al. (2018) employed 16S rRNA gene sequencing to further explore the relationship between the cellulolytic rumen microbiome and feed efficiency in bulls. Their analysis revealed a higher abundance of operational taxonomic units (OTUs) associated with fiber-degrading phyla in efficient RFI bulls, suggesting that the rumen bacterial community was adapted for enhanced fiber degradation, which may influence the feed efficiency phenotype. In a separate study, Elolimy et al. (2018) reported a higher abundance of cellulose-degrading bacteria in the rumen of low RFI beef steers, suggesting their role in improving feed digestibility and energy production in feed-efficient steers. These findings collectively indicate the importance of ruminal fiber-degrading communities in enhancing feed efficiency in cattle, suggesting that selecting for these microbial traits may contribute to the development of more feed-efficient herds fed high-forage diets.

Functional notation

Carbohydrate-active enzymes are essential for breaking down and utilizing complex carbohydrates in cattle feed (Abbott et al., 2018). In this study, we observed differences in the levels of certain enzymes, including glycoside hydrolases, glycoside transferases, and carbohydrate-binding modules, between two groups of Angus bulls. These enzymes are crucial components of the rumen microbiome and have been extensively studied for their biochemical and enzymatic properties (Lombard et al., 2014; Amin et al., 2021). Glycoside hydrolases, also known as glycosidases or glycosyl hydrolases, break glycosidic bonds in complex carbohydrates and play roles in antibacterial defense and pathogenesis in living organisms (Davies and Henrissat, 1995; Sjögren and Collin, 2014).

In our study, we found that the levels of GH5 and GH43 were higher in NegRFI bulls, while GH13 was more abundant in PosRFI bulls. GH5 proteins are one of the largest families and are commonly found in various environments (Henrissat et al., 1989; Dai et al., 2012). GH43 enzymes are known for their ability to break down biomass and are important in degrading plant cell walls (Cantarel et al., 2009; Mewis et al., 2016). Both GH5 and GH43 are involved in breaking down mannan bonds, a type of hemicellulose found in plant cell walls, into usable sugars (Aspeborg et al., 2012; Wang et al., 2013). This breakdown increases fermentable substrates available to rumen microorganisms, potentially increasing VFA production and microbial protein availability (Wang et al., 2019; Hua et al., 2022). The higher levels of GH5 enzymes in NegRFI bulls suggest increased energy substrates and nutrient availability, enhancing ruminal fermentation efficiency and nutrient utilization.

The GH13 family, also known as the α-amylase family, is responsible for rapidly converting starch into glucose and maltose (Bourne and Henrissat, 2001; Stam et al., 2006). Interestingly, despite being fed the same diet as NegRFI bulls, PosRFI bulls had higher levels of GH13, indicating differences in carbohydrate utilization mechanisms between the groups (Lin et al., 2019; Zhao et al., 2022). The relative abundance of GT2 was higher in PosRFI bulls. These enzymes are involved in the biosynthesis of structural molecules from sugar moieties, affecting the overall utilization of carbohydrates for energy substrates and production (Keenleyside et al., 2001).

The relative abundance of CBM86, CBM35, and CBM6 was higher in NegRFI bulls compared to PosRFI bulls. Carbohydrate-binding modules influence carbohydrate binding, degradation, and utilization by facilitating GH binding to carbohydrate structures (Boraston et al., 2004; Ficko-Blean and Boraston, 2006). The greater relative abundance of these enzymes in NegRFI bulls suggests increased enzymatic recognition and hydrolysis, potentially providing additional energy substrates to support better nutrient utilization.

Conclusion

The insights gleaned from this study carry significant implications for beef bull production and progeny, particularly concerning feed efficiency and the observed outcomes in our feed-efficient bulls. Understanding the intricate associations between rumen microbiome composition, host genetics and feed efficiency traits can inform targeted breeding strategies to select

bulls with superior feed efficiency characteristics. Notably, Angus bulls classified as NegRFI exhibit higher gene richness, microbial diversity, and enzymatic functionality, indicative of a thriving microbiome adept at facilitating fermentative metabolism and enhancing nutrient availability. This microbial profile suggests the potential for more efficient energy partitioning towards growth, health, and production in NegRFI bulls. However, we acknowledge that this study does not allow for observing rumen microbiome changes over time, which could arise from shifts in diet and environmental factors. Longitudinal studies would provide a clearer understanding of how ruminal microbial communities evolve and influence feed efficiency under changing conditions. Additionally, broader metagenomic analyses across diverse breeds, age groups, and dietary regimes will provide valuable insights into optimizing beef cattle production systems for enhanced feed efficiency and sustainability.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, sraPRJNA1151032.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee of West Virginia University (IACUC Protocol Number: 2206054350). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TS: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. ET: Data curation, Investigation, Methodology, Writing – review & editing. GT: Data curation, Investigation, Methodology, Writing – review & editing. EF: Writing – review & editing. PF: Writing – review & editing. IO: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2024.1485447/full#supplementary-material

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