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Rumen Fluid Amine/Phenol-Metabolome of Beef Steers with Divergent Residual Feed Intake Phenotype

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Abstract: The amine/phenol-metabolome of rumen fluid was analyzed to identify amino acid metabolism-related biomarkers associated with phenotypic selection for low or high residual feed intake (RFI) in beef cattle. Fourteen beef steers (most feed-efficient (HFE; RFI = −1.89 kg/d, n = 7) and least feed-efficient (LFE; RFI = +2.05 kg/d, n = 7)) were selected from a total of 56 crossbred growing beef steers (average BW = 261 ± 18.5 kg) after a 49-d feeding period in a dry lot equipped with two GrowSafe intake nodes. Rumen fluid samples were collected 4 h after feeding on d 56, 63, and 70 from the HFE and LFE beef steers. Metabolome analysis of the rumen fluid was performed using chemical isotope labeling/liquid chromatography-mass spectrometry to identify all metabolites containing amine/phenol chemical groups, which are mostly amino acid metabolites. A total of 493 metabolites were detected and identified in the rumen fluid. The partial least squares discriminant scores plot showed a slight separation between the two groups of steers, and a total of eight metabolites were found to be differentially abundant (FDR ≤ 0.05). Out of the eight differentially abundant metabolites, four metabolites (isomer 1 of cadaverine, baeocystin, 6-methyladenine, and N(6)-methyllysine) qualified as candidate biomarkers of divergent RFI phenotype based on area under the curve ≥ 0.70. The results of this study revealed that divergent RFI phenotype is associated with alteration in rumen amine/phenol-metabolome of beef steers.

Keywords: residual feed intake; amine/phenol-metabolome; rumen fluid

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1. Introduction

Residual feed intake (RFI), a measure of feed efficiency, is defined as the difference between the expected and actual feed intake of food animals [1]. Residual feed intake is phenotypically favorable because it is independent of animal production measures such as average daily gain, body weight, and carcass traits [2]. Animals with negative (or low) RFI values are considered to be feed-efficient because they consume less feed than their expected intake, whereas animals with positive (or high) RFI values consume more than their expected feed intake to achieve the same growth performance [1]. Not only is selection for low RFI animals gaining popularity amongst beef producers because they eat less per unit of weight gained, but also because low RFI has been positively attributed to less production of methane [3]. With this knowledge, RFI has been deemed as both environmentally and economically important, and several researchers have desired to deduce and establish metabolic biomarkers associated with RFI. [4,5].

Metabolomics has been used in several studies to evaluate the metabolic or nutritional status of animals with divergent RFI phenotypes [5]. Several of these studies have identified amino acid metabolism as an important pathway associated with RFI in ruminants [6,7]. In ruminants, host amino acid metabolism is significantly influenced by ruminal nitrogen metabolism because proteins and AA in feeds are first subject to microbial degradation

in the rumen. To this date, no studies have attempted to determine how the selection for divergent RFI phenotype is associated with the rumen metabolome, with a focus on the metabolites associated with amino acid metabolism. Therefore, the objective of this study was to analyze the rumen amine/phenol-metabolome of crossbred beef steers with divergent RFI phenotype. We hypothesized that beef steers with divergent RFI phenotype would have a different relative abundance of certain metabolites associated with the metabolism of amino acids.

2. Materials and Methods

2.1. Animals, Experimental Design, and Rumen Fluid Sample Collection

Fifty-six (56) crossbred beef steers (average BW = 261 ± 18.5 kg) were fed a total mixed ration diet (Table 1) in a dry lot equipped with GrowSafe intake nodes for 49 d to determine their RFI phenotype. Details of the RFI determination have been reported in our previous study [4]. At the end of the 49-d period, 14 beef steers (most efficient (HFE; RFI = −1.89 kg/d, n = 7) and least efficient (LFE); RFI = +2.05 kg/d, n = 7) were identified and were continued on the same diet for an additional 21 d (representing d 50–70). Rumen fluid samples were collected using an orally administered stomach tube connected to a vacuum pump (Ruminator; profs-products.com (accessed on 14 June 2021), Wittibreut, Bayern, Germany) once weekly for three weeks (d 56, 63, and 70; a total of 42 samples) from the HFE and LFE steers at about 4 hr after morning feeding into 50-mL polypropylene conical bottom tube. Approximately 200 mL of rumen fluid was taken, after discarding the first 150 mL to prevent saliva contamination. The rumen fluid samples were immediately placed on ice after collection, and they were thereafter stored at −80 °C. Amine/phenol-metabolome analysis of all rumen fluid samples (21 samples each from HFE and LFE beef steers) were analyzed using a chemical isotope labeling (CIL)/liquid chromatography-mass spectrometry (LC-MS) technique [8].

Table 1. Ingredient and chemical composition of the basal diet.

Ingredient (%DM)	% of Dietary DM
Corn silage	49.5
Mixed grass hay [1]	47.5
Concentrate supplement [2]	3.0
Nutrient analysis	
Dry matter, %	44.5
Crude protein, %	13.2
Neutral detergent fiber (amylase treated), %	45.9
Acid detergent fiber, %	31.5
Ether extract, %	3.14
Calcium, %	0.66
Phosphorus, %	0.37
Net energy of maintenance, Mcal/kg	1.53
Net energy of gain, Mcal/kg	0.93

[1] Contains a mixture of orchard grass and fescue grass. [2] Contained grain by-product, plant protein products, urea, salt, ground limestone, magnesium sulfate, potassium sulfate, sodium selenite, calcium carbonate, vegetable oil, manganous oxide, vitamin D3 supplement, vitamin A supplement, vitamin E supplement, zinc oxide, basic copper chloride, magnesium chloride, propylene glycol, lecithin, phosphoric acid, ferrous sulfate, calcium iodate, and cobalt carbonate.

2.2. Metabolome Analysis and Data Processing

Amine/phenol-metabolome analysis targets amine- and phenol-containing metabolites, which are mostly amino acid metabolic products [8]. Relative quantification (based on peak ratio values) of all the metabolites was conducted using an Agilent 1100 LC system (Palo Alto, CA) connected to a Bruker Impact HD quadrupole time-of-flight (QTOF) MS. Details of CIL/LC-MS operating conditions and set-up have been described previously [9,10]. A total number of 48 LC-MS data files were generated (six quality control (QC) samples, 21 HFE samples, and 21 LFE samples). The QC sample, prepared from an equal

pooled amount of all samples, was analyzed every eight-sample run to monitor instrument performance. A total of 42 raw LC-MS data files were processed using IsoMS Pro 1.2.14 to remove redundant pairs of adduct ions, dimers, and singlet peaks [11]. The detected peak pairs were identified as metabolites using the CIL and linked identity libraries [12].

2.3. Statistical Analysis

The metabolome data for all 42 samples were analyzed using MetaboAnalyst 5.0 software (https://www.metaboanalyst.ca/ accessed on 12 September 2022). The data were first log-transformed and auto-scaled. Partial least squares discriminant analysis (PLS-DA) scores plot was used to visualize difference between the two groups of beef steers. Differentially abundant metabolites (p-values adjusted for false discovery rate (FDR) ≤ 0.05 [13]) were determined using a volcano plot analysis. The differentially abundant metabolites were further screened using a biomarker analysis based on receiver operating characteristic (ROC) curves [14]. Differentially abundant metabolites having AUC ≥ 0.70 were selected as the metabolites associated with divergent RFI phenotype in this experiment [3].

3. Results

A total of 493 metabolites were detected and identified in the rumen (see Supplementary Table S1). Using the first two principal components with 27.6% and 9.2% of explained variances, the PLS-DA scores plot showed a slight separation between the two groups, thus indicating that divergent RFI phenotype is associated with altered rumen fluid amine/phenol-metabolome of the beef steers (Figure 1). Relative ruminal fluid concentration of four metabolites (adenine, 2-aminomuconic acid, 6-methyladenine, and deoxyadenosine) were greater (FDR ≤ 0.05) in HFE, compared to LFE steers, whereas four metabolites (homoarginine, baeocystin, N(6)-methyllysine, and an isomer of cadaverine (FDR ≤ 0.05) were greater in LFE, compared to HFE steers (Table 2; Figure 2). Out of the eight differentially abundant metabolites, only four metabolites (isomer of cadaverine, baeocystin, 6-methyladenine, and N(6)-methyllysine) with AUC values ≥ 0.70 were identified to be associated with divergent RFI phenotype in this study (Figure 3). The distributions of the four metabolites in LFE and HFE beef steers are shown in Figure 4.

Table 2. Differentially abundant amine/phenol-metabolites in rumen fluid of beef steers with divergent residual feed intake phenotype.

Metabolite	FC	FDR
Adenine	1.60	0.01
2-Aminomuconic acid	1.50	0.01
6-Methyladenine	1.31	0.01
Deoxyadenosine	1.26	0.04
Homoarginine	0.92	0.05
Beaocystin	0.91	0.01
N(6)-methyllysine	0.77	0.02
Isomer 1 of Cadaverine	0.56	0.01

HFE = beef steers with negative residual feed intake; LFE = beef steers with positive residual feed intake. FC: fold change (HFE/LFE). Only metabolites with false discovery rate (FDR) ≤ 0.05 are shown.

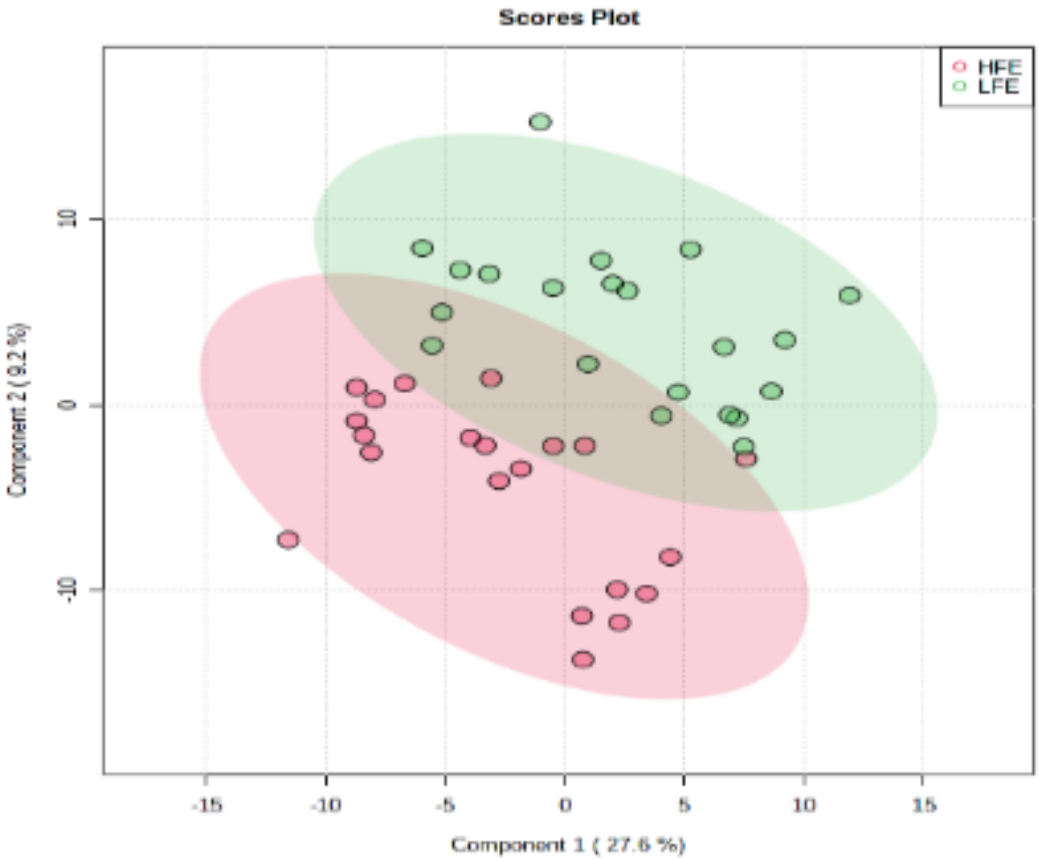

Figure 1. PLS-DA scores plot of rumen metabolome of LFE and HFE steers. HFE = beef steers with negative residual feed intake; LFE = beef steers with positive residual feed intake.

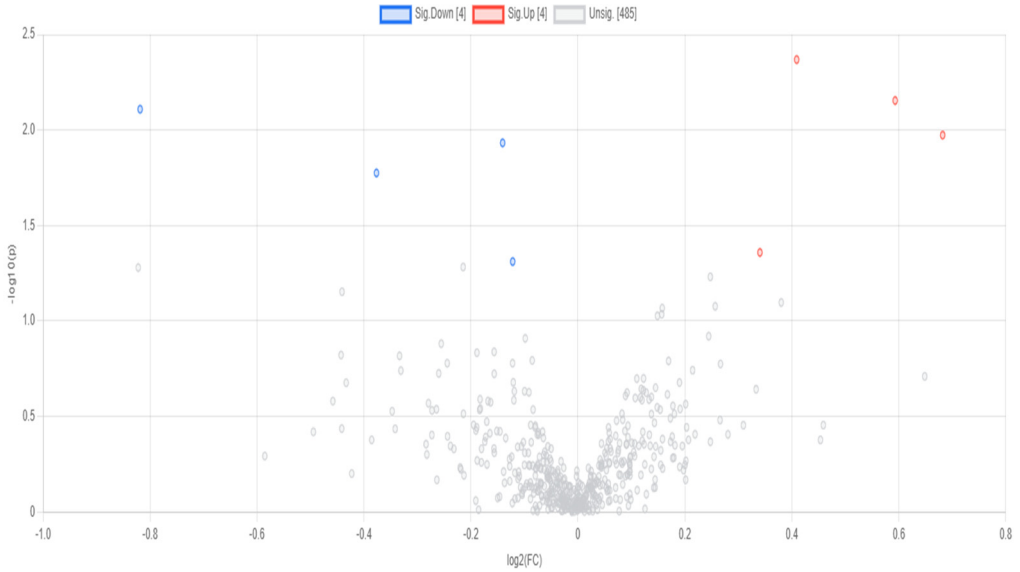

Figure 2. Volcano plot showing the differentially abundant metabolites in rumen fluid. Metabolites with a false discovery ratio of $\leq$0.05 (red or blue) are differentially increased (red dot) or reduced (blue dots) in HFE steers relative to LFE. HFE = beef steers with negative residual feed intake; LFE = beef steers with positive residual feed intake.

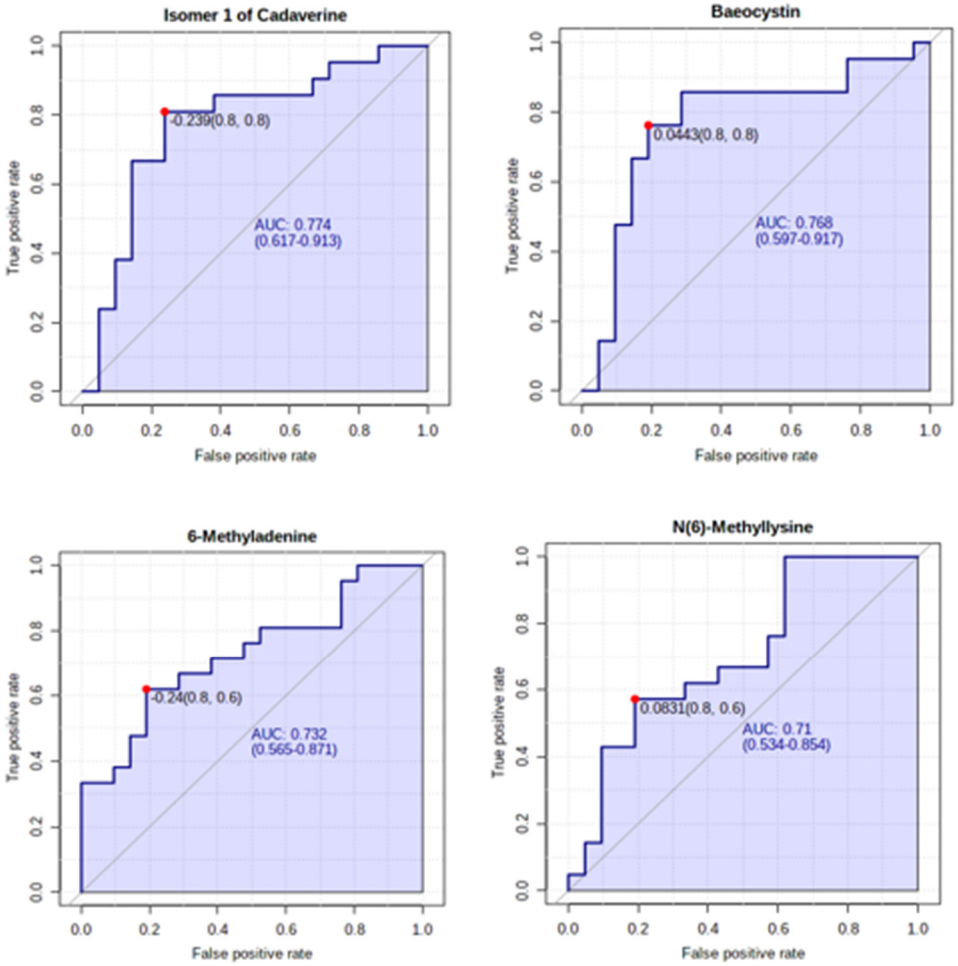

Figure 3. Biomarker analysis of metabolome in rumen fluid. ROC curve analysis of the four metabolites (isomer 1 of cadaverine, baeocystin, 6-methyladenine, N(6)-methyllysine) associated with divergent RFI phenotype.

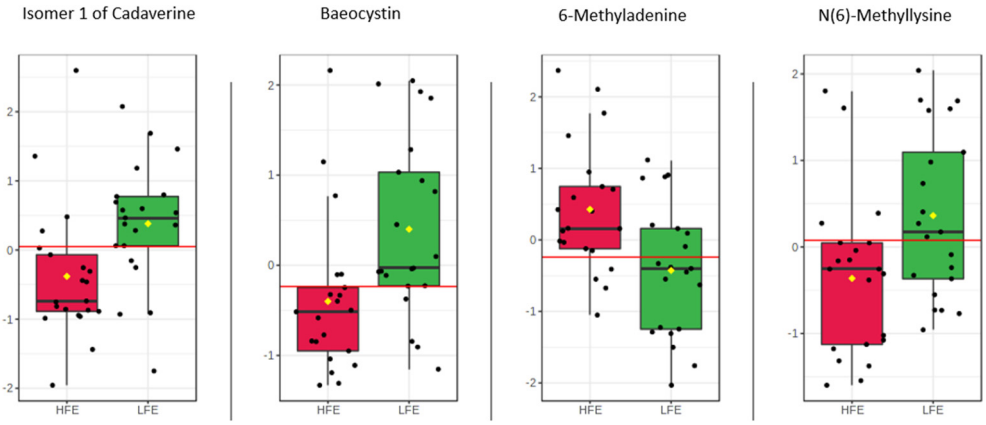

Figure 4. Relative distributions of the metabolites associated with divergent RFI phenotype in beef steers (isomer 1 of cadaverine, baeocystin, 6-methyladenine, N(6)-methyllysine). HFE (red) = beef steers with negative residual feed intake; LFE (green) = beef steers with positive residual feed intake.

4. Discussion

Amino acid metabolism plays a significant role in essential metabolic processes in animal cells and contributes to growth and productivity of animals [15]. In ruminants, host amino acid metabolism is significantly influenced by ruminal nitrogen metabolism because

proteins and AA in feeds are first subject to microbial metabolism in the rumen [16,17]. Altered rumen amine/phenol-metabolome of the beef steers with divergent RFI supports the essential role of ruminal amino acid metabolism to overall animal productivity. Our results agree with results from previous studies that identified amino acid metabolism, or its associated metabolites, as the most significant pathway associated with residual feed intake [4,18]. For instance, Li and Guan (2017) reported an interconnection between ruminal amino acid biochemical pathway and RFI in beef cattle. Similarly, Taiwo et al. (2022) identified four plasma metabolites (methionine, 5-aminopentanoic acid, 2-aminohecanedioic acid, and 4-chlorolysine) associated with amino acid metabolism as the candidate biomarkers associated with phenotypic selection for low or high RFI.

The relative rumen fluid concentration of an isomer of cadaverine and N(6)-methyllysine were greater in LFE steers compared to HFE. Cadaverine is a product of bacterial decarboxylation of lysine that occurs during protein hydrolysis [19]. Cadaverine is an alkane diamine and is one of the four basic polyamines in mammals and humans [20,21]. Polyamines are essential components of mammalian cells and play critical functions in protein synthesis and function [22]. Despite polyamines being involved in physiological processes, polyamines found in high abundance can cause significant toxicity with damages to DNA, protein, tissues, and other cellular components [23]. N(6)-methyllysine is a derivative of lysine methylation [24]. The methylation of lysine functions as a regulator of various effector molecules and is involved in transcriptional regulation, DNA repair, and DNA replication [25,26]. While the biological significance of rumen fluid abundance of isomer 1 of cadaverine and N(6)-methyllysine are unknown, several studies in beef cattle have reported an association between RFI status and plasma lysine concentration [4,6,27]. Lysine is a limiting amino acid in growing beef cattle, and its deficiency can lead to poor growth performance [28,29]. Although the physiological effects of cadaverine and N(6)-methyllysine were not measured in this study, it is reasonable to speculate that lower relative abundance in rumen fluid of LFE steers, compared to HFE steers, could suggest higher ruminal lysine degradation, which can cause lower availability for tissue protein synthesis in LFE steers.

Baeocystin was identified as a candidate metabolite associated with divergent RFI phenotype in this study, and its relative rumen fluid concentration was greater in LFE compared with HFE steers. Baeocystin is a methyl analogue of psilocybin, also referred to as a tryptamine toxin [30,31]. Baeocystin can bind to specific subtypes of 5-HT receptor to produce hallucinogenic effects and is a plausible candidate to induce effects similar to psilocybin [32]. High-level ingestion of tryptamine toxins may be harmful to animals and humans, and while there are minimal studies on the biological significance of baeocystin in animals in reference to feed efficiency, psilocybin, and its analogues, have been reported to cause neurologic effects and tryptamine toxicity in humans' and animals' syndrome [33,34], which might explain reduced efficiency of feed nutrient utilization in LFE steers. 6-Methyladenine is a metabolite involved in regulation of several metabolic processes, such as gene expression, DNA replication, and cell defense against viruses [35,36]. The fact that the relative concentration of 6-methyladenine was greater in HFE compared to LFE steers suggests that there may be increased availability for improved metabolic functions, which are vital for the growth and development of animals.

5. Conclusions

The results of this study revealed that divergent RFI phenotype is associated with altered rumen amine/phenol metabolome. The relative concentrations of four metabolites (6-methyladenine, baeocystin, N(6)-methyllysine, and an isomer of cadaverine) were found to be associated with RFI phenotype. Future studies are needed to validate the roles of these metabolites and how they affect the feed efficiency, amino acid and energy metabolism, and balance of the beef steers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ruminants3010001/s1, Table S1: Relative concentrations of all identified metabolites.

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