Predicting residual feed intake status using rumen microbial profiles in ewe lambs[1]

Melinda J. Ellison,[*,2] Gavin C. Conant,[†] William R. Lamberson, [‡] Kathleen J. Austin,[§] Edward van Kirk,[§] Hannah C. Cunningham,[§] Daniel C. Rule,[§] and Kristi M. Cammack[#]

*Department of Animal and Veterinary Science, Nancy M. Cummings Research, Extension, and Education Center, University of Idaho, Carmen 83462; [†]Department of Biological Sciences, North Carolina State University, Raleigh 27695; [‡]Division of Animal Science, University of Missouri, Columbia 65211; [§]Department of Animal Science, University of Wyoming, Laramie 82071; and [#]Animal Science Department, South Dakota State University, West River Ag Center, Rapid City 57702

ABSTRACT: Including feed efficiency as a trait for selection has gained interest in the sheep industry because it can result in reduced feed inputs or improve stocking rates, both of which translate into increased profitability for the producer. It is of interest whether the feed efficiency status of a testing population of sheep could be predicted using rumen microbial profiles associated with divergent feed efficiency status in a training population of sheep. Two populations of ewes were fed the same diet, and each group was evaluated for feed efficiency. A total of 20 animals in the testing population were selected for prediction assessment using feed efficiency, including the 6 top-ranked, the 6 bottom-ranked, and 8 middle-ranked ewes stratified over the distribution. Rumen fluid samples were collected and DNA was extracted for sequencing. Using a rumen microbial profile associated with diverging feed efficiency created from the training population, multiple discriminant analyses were performed using the DISCRIM procedure of SAS to determine the probability of correctly identifying lambs in the testing population as low, medium, or high feed efficiency using their microbial profiles. A profile of 6 rumen microbial species were used to correctly ($P < 0.001$) predict all testing population ewes into their actual feed efficiency status. A regression analysis using the same microbial profile was used to predict feed efficiency values, which were strongly correlated ($r = 0.71$; $P < 0.001$) with actual feed efficiency values. These results indicate that specific rumen microbial species may play a role in feed efficiency, and that a microbial profile could be used to rank sheep for feed efficiency.

Key words: feed efficiency, RFI prediction, rumen microbiome, sheep

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INTRODUCTION

The rumen microbiome in ruminant livestock is made up of a complex population of microbiota that ferments a wide variety of feedstuffs consumed by the animal into metabolites, principally volatile fatty acids (VFA), that provide approximately 70% of the host's metabolic energy (Bergman, 1990; van Soest, 1994). While diet has been determined to be the primary influence on gastrointestinal (GI) tract microbiota in ruminants (Henderson et al., 2015), changes in GI microbial composition have also been associated with feed efficiency, growth, and milk production (Myer et al., 2017; Schären et al., 2018). In humans, changes in the composition of GI tract microbiota have been associated with several diseases,

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[2]Corresponding author: ellison@uidaho.edu

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including obesity, coronary heart disease, diabetes, and inflammatory bowel disease (Anderson et al., 2009; Benson et al., 2010; Spor et al., 2011).

Including feed efficiency as a trait for selection has gained interest in the sheep industry because it can result in reduced feed inputs or improve stocking ratios, both of which translate into increased profitability for the producer. Residual feed intake (RFI) is a measure of feed efficiency defined as the difference between the actual feed intake and that predicted based on the individual's average daily weight gain (Koch et al., 1963); a lower RFI (i.e., more negative) denotes better feed efficiency. In sheep, RFI was reported to be moderately heritable (h^2 = 0.11–0.30) (Snowder and Van Vleck, 2003; Cammack et al., 2005; Franqois et al., 2007), and because it is independent of growth and mature body size, it has become a commonly applied measure of feed efficiency for livestock (Carberry et al., 2012).

Guan et al. (2008) first proposed differences in rumen microbial composition present in high- versus low-RFI cattle fed a concentrate-based diet, followed by several other studies that confirmed that the microbiota in the rumen was distinguishable between high- and low-efficiency ruminant livestock (Zhou et al., 2009, 2010; Carberry et al., 2012; Myer et al., 2015; Ellison et al., 2017). Although the use of machine-learning and k-nearest neighbors algorithms with large data sets of rumen microbial populations have been described (Shabat et al., 2016; Yao et al., 2016), there has been limited published data evaluating the use of specific rumen microbial abundances to predict feed efficiency status, especially in sheep. Additionally, previous literature describes relationships between high- and low-feed efficient cattle using whole rumen microbiome approaches.

Currently applied feed intake tests can be expensive and time-consuming. However, determining whether a few specific rumen microbial species are associated with divergent feed efficiency could enable prediction of feed efficiency status. This information may then be used with recent technology advancements, such as handheld DNA sequencing (i.e., MinION, Oxford Nanopore Technologies). Collectively, these techniques may provide producers with a chute-side method that is faster and more economical for determining feed efficiency. The objective of this study was to determine whether the feed efficiency status of Targhee ewes could be predicted using a profile of rumen microbiota previously found to be associated with divergent feed efficiency status in a training population of similar ewes when fed the same diet.

MATERIALS AND METHODS

Testing Population: Animals and Diet

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. Growing Targhee ewe lambs (n = 59; initial body weight = 45.8 ± 2.5 kg) were fed a forage-based pelleted diet (67.7% alfalfa and 27.5% wheat middlings; 18.6% CP, 42.5% NDF, 2.31 Mcal ME/kg, dry matter basis). Individual feed intake was measured using the GrowSafe System (Airdrie, Alberta, Canada) for a 70 d trial period. Two-day average initial and final body weight (BW) were obtained to calculate average daily gain (ADG), and a mid-BW to calculate metabolic ($BW^{0.75}$) midweight (MMWT). From these data, RFI was calculated as the deviation of true feed intake from expected feed intake. Expected feed intake was determined by regressing actual feed intake on ADG and MMWT (Cammack et al., 2005). RFI estimates were used to rank ewes for efficiency. In total, 20 animals were selected for further study, including the 6 top-ranked ewes for RFI (1–6) and the 6 bottom RFI ranked ewes (54–59). Additionally, 8 middle ranked RFI ewes stratified over the RFI distribution (ranks 6, 11, 16, 21, 26, 39, 44, 49, and 54) were selected. The selected ewes represented the greatest (n = 6) and lowest (n = 6) ranked animals for feed efficiency and a subset that represented the moderately ranked ewes (n = 8), to characterize the overall distribution of RFI values (RFI = −1.4 to +0.8 kg/d) within the population. Lambs were classified as high (**L-RFI**; RFI = −0.5 to −1.4 kg/d; n = 6), medium (**M-RFI**; RFI = −0.4 to +0.4 kg/d; n = 8) or low (**H-RFI**; RFI = +0.5 to +0.8 kg/d; n = 6) feed efficiency. Unfiltered rumen samples (≥30.0 mL; samples included fluid and solid fraction) were collected from the selected ewes at the end of the feeding trial using a tygon tube (length: 1 m, diameter: 1.5 cm) positioned through the mouth, down the esophagus, and into the rumen and a dosing syringe (400 mL) for suction. Samples were then allocated in triplicate into 2-mL tubes for DNA extraction, snap-frozen, and stored at −80 °C until processing. The remaining rumen fluid was retained for VFA analysis and was stored at −20 °C until processing.

VFA Analysis

Preparation of samples for VFA analysis was conducted according to Goetsch and Galyean (1983). Thawed rumen fluid samples (≥10 mL) from the selected ewes were centrifuged at 3,000 × g for 10 min

and supernatant was added to a solution containing 25% metaphosphoric acid that contained 2-ethyl butyric acid (2-EB) as internal standard (2.0 mg/mL) such that the ratio of the volume of rumen fluid to metaphosphoric acid was 5:1. Samples were incubated on ice for 30 min and centrifuged at 3,000 × *g* for 30 min. One milliliter of supernatant was transferred to automatic sampler vials for analysis by gas–liquid chromatography. Detector response factors for acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate were determined for 2-EB for calculation of molar percentages of each VFA. Volatile fatty acid concentrations were determined using an Agilent 6890 gas chromatograph with Agilent ChemStation software (Agilent Technologies, Santa Clara, CA) equipped with a Supelco Nukol (0.25 µm thickness; Sigma–Aldrich Corp., St. Louis, MO), 15 m × 0.53 mm capillary column. Detector and injector temperatures were 250 °C. Initial oven temperature was 80 °C and was increased at a rate of 8 °C per min to 150 °C. Hydrogen (H_2) was used as carrier gas at a flow rate of 20 mL/min.

DNA Extraction from Rumen Fluid

DNA was extracted from rumen fluid of the selected ewes using methods detailed by Yu and Morrison (2004). Zirconia (0.3 g of 0.1 mm) and silicon (0.1 g of 0.5 mm) beads and 1 mL lysis buffer were added to thawed rumen fluid samples (250 mg). Samples were homogenized using a Mini-Beadbeater-8 at maximum speed for 3 min, incubated at 70 °C for 15 min with gentle mixing every 5 min, and centrifuged at 16,000 × *g* and 4 °C for 5 min. Supernatant was transferred to new 2-mL flat cap tubes, and fresh lysis buffer (300 µL) was added to the pelleted beads. The homogenization, incubation, and centrifugation steps were repeated on the remaining bead pellet, and supernatants were pooled. One volume of isopropanol was added to the supernatants followed by centrifugation at 4 °C for 15 min at 16,000 × *g* to precipitate the DNA. Final purification for removal of RNA and proteins was completed using the protocol of the QIAamp DNA Stool Mini Kit (Qiagen, Santa Clarita, CA). Samples of DNA were then quantified on the NanoDrop spectrophotometer (NanoDrop, Wilmington, DE) and determined to have acceptable quality using the manufacturer prescribed standard of the A260/280 ratio ≥1.8.

Microbial Library Preparation and Sequencing

Extracted DNA (10 µg) was sent to the University of Missouri (Columbia) DNA Core Facility for high-throughput sequencing. The library was constructed following the manufacturer's protocol with reagents supplied in Illumina's TruSeq DNA PCR-Free sample preparation kit (#FC-121-3001). Briefly, 1 µg of genomic DNA was sheared using standard Covaris methods to generate average fragmented sizes of 350 bp. The resulting 3′ and 5′ overhangs were converted to blunt ends by an end repair reaction which uses a 3′ to 5′ exonuclease activity and polymerase activity. The desired size of the fragment (~550 bp) was selected by sample purification beads (AMPure XP). A single adenosine nucleotide was added to the 3′ ends of the blunt fragment followed by the ligation of Illumina indexed paired-end adapters. The adaptor-ligated library was purified twice with sample purification beads. The purified library was quantified with a Qubit assay and library fragment size confirmed by Fragment Analyzer (Advanced Analytical Technologies, Inc.). The library samples were diluted and sequenced according to Illumina's standard sequencing protocol for the HiSeq.

The resulting 20 libraries were sequenced on an Illumina HighSeq 2500 platform using a PE100 run. A total of 3 flowcells were run, with 5 libraries per flowcell lane and each sample was stratified across every flowcell. The resulting 100 base-pair, paired-end reads from each lane were pooled within samples and then quality trimmed by truncating each read after the first run of 3 bases using a Phred quality score < 15, and omitting reads with < 85 base pairs or a quality score of <25. The filtered read pairs were compared with a database of 27,000 known 16S rDNA genes using the Bowtie reference-based assembly tool (Langmead et al. 2012) (Johns Hopkins, Baltimore, MD), as previously described by Ellison et al. (2014). Operational taxonomic units (OTUs) were inferred using a graph analysis of all database sequences, where pairs of sequences in the graph were connected if their sequence identity after Needleman–Wunch alignment was ≥97% (Needleman and Wunsch, 1970; Truong et al. 2014). With this structure, OTUs were defined to be the connected components obtained from this graph using single-linkage clustering (Ellison et al., 2014). A read pair was considered to derive from a particular OTU if both reads matched to sequences from that OTU and *only* that OTU with ≥97% identity.

Training Population: Predictive Rumen Microbial Profile

Briefly, 1 year prior to the testing population trial, an initial trial consisted of Targhee ewe lambs

(n = 78, initial BW = 55.7 ± 1.2 kg) similar in age and body composition, and from the same producer flock as the testing population. Those lambs were used as a "training" population to determine rumen microbial characteristics associated with divergence for feed efficiency (H-RFI vs. L-RFI). The ewe lambs in the training population were fed the same diet as the testing population and were performance tested for 70 d for RFI estimation. The ewe lambs were ranked on RFI, and the rumen microbial profiles from lowest ranking RFI ewes (n = 8; RFI = −0.4 to −0.7 kg/d) and the highest ranking RFI ewes (n = 8; RFI = 0.3 to 0.9 kg/d) were compared to determine differences associated with divergence for RFI classification (L-RFI vs. H-RFI) that were then used to predict RFI classification in the testing population described above.

Because differences were observed in a number of microbial characteristics in the training population, several profiles were considered for determining predicted RFI status in the testing population:

1. *Total microbial species and abundance profile.* First, the total number of microbial species and the total microbial abundance were used individually and then in combination to predict feed efficiency, both of which were previously reported to be greater in low efficiency (H-RFI) Targhee ewes (data not shown).
2. *Species-level profile.* Finally, the species-level profile included *Schwartzia succinivorans*, which was of greater proportional abundance in low efficiency (H-RFI) Targhee ewe lambs, and *Prevotella genomospecies, Pseudobutyrivibrio ruminis,* and *Treponema maltophilum*, all of which were of greater proportional abundance in high-efficiency (L-RFI) Targhee ewe lambs.

Training Population: Statistical Analysis

A mixed model was used to assess the effects of feed efficiency status on BW characteristics, average daily feed intake (ADFI), ADG, and gain-to-feed ratio (G:F) and on VFA concentrations for RFI-selected ewes (n = 16). In these models, RFI class was assumed to be a fixed effect and animals were a random effect.

Taxa, for further analysis in the testing population, were initially screened by retaining only those present in 80% of the training animals. For further screening, the relative abundance of these remaining species was then examined for significance across RFI classes using a generalized linear mixed model assuming a beta distribution with a logit-link function (Stroup, 2014). Only those species demonstrating a significance greater than P = 0.05 were considered for further analysis in the testing population. An analogous methodology was repeated at the genus level; however, there were no differences (P > 0.05) between H- and L-RFI ewes at the genus level. Therefore, only species-level profiles were included in further analysis. All analyses were carried out using SAS 9.4 (Proc Mixed; Proc Glimmix).

Testing Population: Statistical Analysis

A mixed model was used to determine the effects of feed efficiency status on BW characteristics, ADFI, ADG, and G:F, and on VFA concentrations for the selected ewes (n = 20) in the testing population. In these models, RFI class was assumed to be a fixed effect and animals were a random effect. A multiple discriminant analysis using the DISCRIM procedure of SAS was used to determine the probability of correctly identifying lambs as L-RFI, M-RFI or H-RFI based on the rumen microbial profiles described above that were established in the training population. For the species-level profile, the multiple discriminant analysis initially included all the significant species-level taxa, and then each of the taxa was removed stepwise (i.e., one-by-one) to determine whether its removal would improve or decrease the probability of correctly classifying the lambs. Those taxa that did not decrease the effectiveness of the classification when removed from the model were then removed 2 or more at a time until the removal of any remaining taxa negatively impacted correct classification. Finally, RFI was regressed on the proportional abundances of the 6 taxa from the final species-level profile using the GLM procedure of SAS to determine predicted RFI based on individual proportional abundances of these taxa. The CORR procedure of SAS was used to determine the correlation between actual and predicted RFI values and between actual and predicted RFI classifications.

RESULTS

Animal Performance: Testing Population

Performance traits are listed in Table 1 for selected ewes classified for feed efficiency in the training population (H-RFI or L-RFI) and in the testing population (H-RFI, M-RFI, or L-RFI). The RFI values in the training population ranged from

Table 1. Least-squares means of performance traits in ewe lambs divergent for residual feed intake (RFI)

	Feed efficiency status							
	Training population		Testing population				*P*-values	
	H-RFI[1]	L-RFI[2]	H-RFI	M-RFI[3]	L-RFI	SEM	Training	Testing
Performance trait								
RFI, kg/d	0.6	−0.5	0.6[a]	0.0[b]	−0.8[c]	0.09	<0.001	<0.001
ADG[4], kg/d	0.3	0.3	0.3	0.3	0.3	0.02	0.309	0.639
ADFI[5], kg/d	4.0	2.8	3.6[a]	2.8[b]	2.1[c]	0.11	<0.001	<0.001
G:F[6], kg/kg	0.08	0.10	0.08[b]	0.09[b]	0.12[a]	0.01	<0.001	0.006
MMWT[7], kg	23.8	23.1	21.1	19.8	20.9	0.83	0.343	0.492
D 0 BW[8], kg	56.8	54.8	48.8	45.2	48.8	3.14	0.457	0.595
D 35 BW, kg	68.4	65.6	60.2	55.3	58.0	3.12	0.332	0.505
D 70 BW, kg	78.6	75.2	68.4	63.0	66.7	3.16	0.282	0.425
Volatile fatty acid (molar %)								
Acetate	65.0	65.5	67.5	66.7	65.9	0.01	0.650	0.229
Propionate	15.9	15.6	14.2	14.6	15.3	0.01	0.684	0.203
Butyrate	14.4	13.8	13.1	13.5	13.9	0.01	0.468	0.675
Valerate	1.5	1.4	1.5	1.5	1.5	0.01	0.461	0.986
Isobutyrate	1.4	1.6	1.7	1.7	1.5	0.01	0.098	0.753
Isovalerate	1.8	2.1	2.0	2.1	1.8	0.01	0.155	0.757

[1]H-RFI = high RFI (low feed efficiency status; RFI = +0.5 to +0.8 kg/d; training population, *n* = 8; testing population, *n* = 6).
[2]L-RFI = low RFI (high feed efficiency status; RFI = −0.5 to −1.4 kg/d; training population, *n* = 8; testing population, *n* = 6).
[3]M-RFI = moderate RFI (moderate feed efficiency status; RFI = −0.4 to +0.4 kg/d; testing population, *n* = 8).
[4]ADG = average daily gain.
[5]ADFI = average daily feed intake.
[6]G:F = gain to feed ratio (ADG/ADFI).
[7]MMWT = metabolic mid-weight (((D 0 BW + D 70 BW)/2)$^{0.75}$).
[8]BW = body weight.

0.9 to −0.7 kg/d and the average RFI for H-RFI ewes was 0.6 ± 0.05 kg/d, and for L-RFI ewes was −0.5 ± 0.05 kg/d. The RFI values in the testing population ranged from 0.8 to −1.4 kg/d overall, and the average RFI for H-RFI ewes was 0.6 ± 0.09 kg/d, for M-RFI ewes was 0.0 ± 0.08 kg/d and for L-RFI ewes was −0.8 ± 0.09 kg/d. As expected, in both the training and the testing populations, ADG did not differ ($P \geq 0.309$) among ewes differing in feed efficiency, and H-RFI ewes had greater ADFI ($P < 0.001$) and lower G:F ($P \leq 0.006$) than L-RFI ewes, with M-RFI ewes intermediate for ADFI in the testing population. Furthermore, there were no differences ($P \geq 0.282$) in MMWT, day 0 BW, day 35 BW, or day 70 BW across RFI statuses in both the training and testing populations.

Volatile Fatty Acids

For both the training and testing populations, as expected in ewes fed an alfalfa-based diet, acetate was the most abundant VFA in each lamb regardless of feed efficiency status, followed by propionate and butyrate (Table 1). While there were no differences ($P \geq 0.155$) in acetate, propionate, butyrate, valerate, or isovalerate concentrations between L-RFI and H-RFI training population lambs, isobutyrate concentration tended ($P = 0.098$) to be greater in L-RFI ewes compared with H-RFI ewes. Additionally, there were no differences ($P \geq 0.203$) in acetate, propionate, butyrate, valerate, isobutyrate, or isovalerate concentrations among H-RFI, M-RFI, or L-RFI testing population ewes.

Total Microbial Species and Abundance Profile

Using the total number of individual microbial species present in each rumen sample, total microbial abundance, or a combination of the 2 in the multiple discriminant analysis did not result in correct identification ($P \geq 0.10$) of feed efficiency status (data not shown). The total number of individual microbial species misclassified ($P = 0.840$) 5 of the 6 L-RFI ewes into M-RFI and all 6 of the H-RFI ewes into either M-RFI ($n = 5$) or L-RFI ($n = 1$); only one of the L-RFI was correctly classified, but all 8 of the M-RFI ewes were correctly classified. The total microbial abundance analysis misclassified ($P = 0.555$) all 6 of the H-RFI and 5 of the 6 L-RFI ewes into M-RFI, and one of the L-RFI ewes into the H-RFI group; however, again all 8 of the M-RFI ewes were correctly identified

as M-RFI. Using both the total number of individual microbial species and total microbial abundance together, 14 of the 20 ewes were misclassified ($P = 0.478$).

Species-level Profile

The initial species-level profile was made up of all 5 species that were identified in the testing population to differ by feed efficiency status (Table 2), including *Prevotella genomospecies, Pseudobutyrivibrio ruminis, Schwartzia succinivorans*, and *Treponema maltophilum.* Proportional abundance of rumen microbial taxa for the testing population is depicted in Table 3. There were 5 *Prevotella genomospecies,* 2 *Pseudobutyrivibrio ruminis* subspecies, *Schwartzia succinivorans*, and *Treponema maltopmhilum* present in at least one of the selected ewes in the testing population. When all 9 of the species and subspecies were included in the profile, all 20 of the ewes were correctly ($P < 0.001$) classified into their actual feed efficiency status (Table 4).

Subspecies of *Prevotella genomospecies* and *Pseudobutyrivibrio ruminis* are labeled numerically in Table 3 for clarification. When at least 3 of the 5 *Prevotella genomospecies* were included in the original profile, regardless of which combination of 3, all of the selected animals were correctly identified by feed efficiency status. When only 2 *Prevotella genomospecies* were included, several combinations decreased the probability of correctly classifying the ewes; however, when *P. genomospecies* (1) was combined with *P. genomospecies* (2) or when *P. genomospecies* (2) was combined with *P. genomospecies* (5), all 20 of the ewes were correctly classified ($P < 0.001$ and $P = 0.020$, respectively) into their actual feed efficiency class. For the final profile, *P. genomospecies* (1) and *P. genomospecies* (2) were included because this combination resulted in the greatest probability of correct classification.

There were 2 *Pseudobutyrivibrio ruminis* subspecies present in at least one of the selected animals. Removing both subspecies of *Pseudobutyrivibrio ruminis* at the same time resulted in the misclassification ($P = 0.20$) of 7 ewes, including 3 of the M-RFI animals misclassified as L-RFI ($n = 1$) or H-RFI ($n = 2$), as well as 2 L-RFI and 2 H-RFI misclassified as M-RFI. Although removing *Pseudobutyrivibrio ruminis* (1) had no negative effect ($P = 0.037$) on the classification, the probability of success for most of the animals decreased. The removal of *Pseudobutyrivibrio ruminis* (2) resulted in the misclassification ($P = 0.42$) of 9 ewes. Therefore, both *Pseudobutyrivibrio ruminis* species remained in the profile model.

The removal of *Treponema maltophilum* from the profile misclassified ($P = 0.004$) one M-RFI as L-RFI. Finally, removing *Schwartzia succinivorans* had no negative effect ($P < 0.001$) on the correct classification of ewes into RFI category, but it did slightly decrease the probability of success for some of the ewes. Therefore, species included in the final profile to predict RFI classification were *P genomospecies* (1) and (2), *Pseudobutyrivibrio ruminis* (1) and (2), *S. succinivorans* and *T. maltophilum.* From this profile, all 20 of the ewes were correctly ($P < 0.001$) classified into their actual feed efficiency status (Table 5) with 100% probability for all 20 animals.

Predicted RFI

The predicted RFI values (i.e., those estimated from a regression analysis using relative microbial abundances of species in the profile) had a smaller range of RFI values (RFI = +0.71 to −0.89 kg/d) than the actual RFI values (RFI = +0.77 to −1.36 kg/d) in the testing population. However, there was a strong positive correlation ($r = 0.71$; $P < 0.001$) between actual and predicted RFI values based on proportional abundances of the 6 taxa from the final species-level profile (Tables 6 and 7).

When the predicted RFI values were further classified as high (predicted L-RFI; $n = 6$;

Table 2. Least-squares means of proportional abundance of statistically different taxa in training population ewe lambs divergent for residual feed intake (RFI)

	Feed efficiency status			
	H-RFI[1]	L-RFI[2]	SEM	*P*-value
Prevotella genomospecies	0.14%	0.40%	0.08	0.049
Pseudobutyrivibrio ruminis	0.04%	0.14%	0.03	0.025
Schwartzia succinivorans	0.89%	0.33%	0.17	0.018
Treponema maltophilum	0.02%	0.12%	0.02	0.011

[1]H-RFI = high RFI (low feed efficiency status).

[2]L-RFI = low RFI (high feed efficiency status).

Table 3. Proportional abundance[1] of rumen microbial taxa in the testing population of Targhee ewe lambs ranked for residual feed intake (RFI)

	RFI rank (%)																			
	H-RFI[2]						M-RFI[3]								L-RFI[4]					
	1	2	3	4	5	6	11	16	21	26	34	39	44	49	54	55	56	57	58	59
*Prevotella genomospecies (1)**	0.58	11.82	6.41	9.25	0.78	4.92	1.43	0.36	6.96	8.87	0.82	0.32	14.85	9.83	3.80	1.53	0.37	2.44	6.70	0.67
*Prevotella genomospecies (2)**	4.34	0.30	1.87	1.08	2.84	1.40	2.77	2.84	1.64	0.00	0.82	1.29	0.35	0.91	0.24	1.40	2.00	2.06	0.40	2.00
*Prevotella genomospecies (3)**	0.15	2.79	1.74	3.87	0.29	0.50	0.57	0.00	1.09	2.09	0.00	0.16	2.19	1.95	0.87	0.28	0.12	0.75	1.31	0.11
*Prevotella genomospecies (4)**	0.72	0.06	0.53	0.43	1.57	1.00	0.57	0.00	0.96	0.35	0.60	0.60	0.00	0.56	1.11	0.84	0.98	0.94	0.00	0.56
*Prevotella genomospecies (5)**	0.15	0.12	0.00	0.00	0.00	0.00	0.95	0.24	0.27	0.00	0.27	0.00	0.07	0.07	0.08	0.00	0.37	0.19	0.00	0.44
*Pseudobutyrivibrio ruminis (1)**	0.15	0.12	0.53	0.00	0.10	0.00	0.19	0.00	0.00	0.17	0.14	0.00	0.28	0.21	0.32	0.42	0.37	0.19	0.13	0.00
*Pseudobutyrivibrio ruminis (2)**	0.00	0.06	0.13	0.00	0.10	0.10	0.00	0.24	0.00	0.00	0.00	0.00	0.14	0.00	0.32	0.28	0.24	0.00	0.00	0.00
Schwartzia succinivorans^	0.43	0.18	0.00	0.00	0.00	0.00	0.38	0.36	0.00	0.52	0.27	0.00	0.28	0.28	0.24	0.56	0.24	0.18	0.13	0.22
*Treponema maltophilum**	0.00	0.18	0.00	0.43	0.00	0.20	0.00	0.24	0.00	0.00	0.00	0.16	0.00	0.07	0.08	0.13	0.00	0.00	0.13	0.00

[1]A proportional abundance equal to zero may have been below detection.

[2]H-RFI = high RFI (low feed efficiency status).

[3]M-RFI = moderate RFI (moderate feed efficiency status).

[4]L-RFI = low RFI (high feed efficiency status).

*Taxa of greater proportional abundance in high feed efficiency ewes (training population).

^Taxa of greater proportional abundance in low feed efficiency ewes (training population).

Table 4. Percent of testing population ewe lambs accurately categorized ($P < 0.001$) into residual feed intake (RFI) class by a multiple discriminant analysis using a species-level rumen microbe profile[1] that differed between high- and low-RFI Targhee ewe lambs in the training population.

	Predicted RFI class, %		
Actual RFI Class	H-RFI[2]	M-RFI[3]	L-RFI[4]
H-RFI	**100.00**	0.00	0.00
M-RFI	0.00	**100.00**	0.00
L-RFI	0.00	0.00	**100.00**

[1]Profile included 5 *Prevotella genomospecies*, 2 subspecies of *Pseudobutyrivibrio ruminis, Schwartzia succinivorans*, and *Treponema maltophilum*.

[2]H-RFI = high RFI (low feed efficiency status; RFI = +0.5 to +0.8 kg/d; n = 6).

[3]M-RFI = moderate RFI (moderate feed efficiency status; RFI = −0.4 to +0.4 kg/d; n = 8).

[4]L-RFI = low RFI (high feed efficiency status; RFI = −0.5 to −1.4 kg/d; n = 6).

Table 5. Percent of testing population ewe lambs accurately categorized ($P < 0.001$) into residual feed intake (RFI) class by multiple discriminant analysis using a species-level rumen microbe profile[1] that differed between high- and low-RFI Targhee ewe lambs in the training population

	Predicted RFI class, %		
Actual RFI Class	H-RFI[2]	M-RFI[3]	L-RFI[4]
H-RFI	**100.00**	0.00	0.00
M-RFI	0.00	**100.00**	0.00
L-RFI	0.00	0.00	**100.00**

[1]Profile included 2 *P. genomospecies*, 2 subspecies of *Pseudobutyrivibrio ruminis*, *Schwartzia succinivorans* and *Treponema maltophilum*.

[2]H-RFI = high RFI (low feed efficiency status; RFI = +0.5 to +0.8 kg/d; n = 6).

[3]M-RFI = moderate RFI (moderate feed efficiency status; RFI = −0.4 to +0.4 kg/d; n = 8).

[4]L-RFI = low RFI (high feed efficiency status; RFI = −0.5 to −1.4 kg/d; n = 6).

RFI = −0.51 to −0.89 kg/d), moderate (predicted M-RFI; $n = 8$; RFI = −0.50 to +0.31 kg/d) or low (predicted H-RFI; $n = 6$; RFI = +0.32 to +0.71 kg/d) feed efficiency, the correlation tended to be moderately correlated ($r = 0.42$; $P = 0.068$; Tables 6 and 7). Of the 6 actual L-RFI lambs, 3 were correctly ranked into predicted L-RFI and 3 were ranked into predicted M-RFI (Table 7). Of the 8 actual M-RFI lambs, 5 were correctly ranked into predicted M-RFI, 2 were ranked into predicted H-RFI, and one was ranked into predicted L-RFI. Of the 6 H-RFI lambs, only one was correctly ranked into predicted H-RFI, and the remaining 5 were ranked as predicted M-RFI. In total, 9 lambs were correctly ranked into their appropriate predicted RFI class, 10 lambs were only misclassified by one class (i.e., L-RFI into M-RFI, M-RFI into H-RFI, etc.), and none of the lambs were incorrectly classified from H-RFI into L-RFI.

DISCUSSION

Prediction Model

To date, rumen microbiome prediction models have described shifts in whole rumen microbiome composition when comparing high- and low-efficiency cattle. Cattle with lower feed efficiency have been suggested to have a greater overall abundance of species and increased microbial diversity compared with more efficient cattle when analyzed using the k-nearest neighbors algorithm (Shabat et al., 2016). In this trial, a species-specific rumen microbial profile using a multiple discriminant analysis successfully predicted RFI classification (H-RFI, M-RFI, or L-RFI) in ewe lambs. Furthermore, the predicted RFI values that were derived from the regression analysis using the same species-specific rumen microbial profile were strongly correlated ($r = 0.71$; $P < 0.001$) with the actual RFI values. These results indicate that specific rumen microbial species may play a role in feed efficiency, and that the microbial profile could be used to rank sheep for RFI. Furthermore, the accuracy of these results suggests that selection for L-RFI, or high feed efficient, ewe lambs may be possible, and with no negative influence on growth.

Confirmation of these results in a larger group of Targhee ewe lambs is necessary to determine whether the species-level multiple discriminant analysis and predicted RFI regression analysis would be successful in correctly identifying feed efficiency class in another trial. It is also important to note that similar results may not be achieved in lambs consuming different diets. Furthermore, differences in GI microbial composition have been identified among ruminant breeds (Li et al., 2019) and species (O'Donnell et al., 2017); therefore, further research is needed to determine how this prediction model may translate across ruminant breeds and species.

Microbial Profile

Ewes were correctly categorized into their respective RFI classification using the species-level

Table 6. Correlations between actual residual feed intake (RFI) values and categories and predicted RFI values and categories based on relative abundances of 6 rumen microbial species[1] in the testing population of ewe lambs.

	Actual RFI	Predicted RFI	Actual RFI class	Predicted RFI class
Actual RFI	1.00	0.71[2]		
Predicted RFI	0.71[2]	1.00		
Actual RFI class			1.00	0.42[3]
Predicted RFI c lass			0.42[3]	1.00

[1]Microbial profile included 2 *P. genomospecies*, 2 subspecies of *Pseudobutyrivibrio ruminis*, *Schwartzia succinivorans* and *Treponema maltophilum*.
[2]$P < 0.001$,
[3]$P = 0.068$.

Table 7. Actual and predicted residual feed intake (RFI) and RFI class in the testing population of ewe lambs.

RFI Rank	Actual RFI	Predicted RFI	Actual RFI Class	Predicted RFI Class[4]
1	−1.36	−0.89	L-RFI[1]	L-RFI*[5]
2	−0.70	−0.13	L-RFI	M-RFI[6]
3	−0.69	−0.39	L-RFI	L-RFI*
4	−0.65	−0.73	L-RFI	L-RFI*
5	−0.62	−0.29	L-RFI	M-RFI
6	−0.62	−0.18	L-RFI	M-RFI
11	−0.37	−0.27	M-RFI[2]	M-RFI*
16	−0.16	−0.35	M-RFI	L-RFI
21	−0.07	−0.20	M-RFI	M-RFI*
26	−0.00	0.54	M-RFI	H-RFI[7]
34	0.12	0.65	M-RFI	H-RFI
39	0.18	0.19	M-RFI	M-RFI*
44	0.27	−0.08	M-RFI	M-RFI*
49	0.36	−0.15	M-RFI	M-RFI*
54	0.47	0.71	H-RFI[3]	H-RFI*
55	0.50	0.30	H-RFI	M-RFI
56	0.54	0.22	H-RFI	M-RFI
57	0.60	−0.09	H-RFI	M-RFI
58	0.64	0.19	H-RFI	M-RFI
59	0.77	0.15	H-RFI	M-RFI

[1]Actual H-RFI = high RFI (low feed efficiency status; RFI = +0.5 to +0.8 kg/d; $n = 6$).
[2]Actual M-RFI = moderate RFI (moderate feed efficiency status; RFI = −0.4 to +0.4 kg/d; $n = 8$).
[3]Actual L-RFI = low RFI (high feed efficiency status; RFI = −0.5 to −1.4 kg/d; $n = 6$).
[4]Predicted RFI were classified based on a smaller range of RFI values due to a smaller group of animals ($n = 20$) than Actual RFI ($n = 59$). The H-RFI, M-RFI, and L-RFI classes were determined using mean ± SD from Predicted RFI values.
[5]Predicted L-RFI = low RFI (high feed efficiency status; RFI = −0.51 to −0.89 kg/d; $n = 6$).
[6]Predicted M-RFI = moderate RFI (moderate feed efficiency status; RFI = −0.50 to +0.31 kg/d; $n = 8$).
[7]Predicted H-RFI = high RFI (low feed efficiency status; RFI = +0.32 to +0.71 kg/d; $n = 6$).
*Predicted RFI class was the same as actual RFI class ($n = 9$).

rumen microbial profile developed from the initial training population, which included 2 *P. genomospecies,* 2 subspecies of *Pseudobutyrivibrio ruminis, S. succinivorans*, and *T. maltophilum*. *Prevotella* species are, in general, predominant bacteria in the rumen and are especially diverse because they can degrade starch, fiber, and protein (Carberry et al., 2012). *Schwartzia succinivorans* are a known succinate-utilizing species (Van Gylswyk et al., 1997). The roles that *Prevotella genomospecies* and *S. succinivorans* may play in feed efficiency are unclear.

Pseudobutyrivibrio ruminis have been described as closely related to *Butyrivibrio fibrisolvens*, which are one of the predominant hemicellulose degraders in the rumen (Grilli et al., 2013; Puniya et al., 2015). Bacteria that are similar to *Butyrivibrio* tend to make up a large portion of rumen microbes, especially in wild ruminants, consuming poor-quality feeds (Orpin et al., 1985; Forster et al., 1996). The

ability to degrade poorer quality feedstuffs may help explain the importance that *Pseudobutyrivibrio ruminis* plays in feed efficiency in sheep due to its ability to degrade more complex fibers in the diet that may not otherwise be utilized.

While members of the genera *Treponema* are known to use carbohydrates and amino acids for energy sources in the rumen (Willey et al., 2008), several species in the *Treponema* genus have also been suggested to play a pathogenic role. *Treponema maltophilum* and other *Treponema* species have been associated with both bovine digital dermatitis lesions and severe virulent ovine foot rot lesions, infectious diseases that cause lameness (Demirkan et al., 2001; Zinicola et al., 2015). Fecal and ruminal abundance of *Treponema maltophilum* and 5 other *Treponema species* were greater in cattle diagnosed with bovine digital dermatitis lesions (Zinicola et al., 2015). It is difficult to explain the increased abundance of *Treponema maltophilum* in high-efficiency lambs; however, it may be that it is a natural flora within the rumen, but when living in soil or excreted feces, becomes opportunistic to susceptible abrasions or lesions on the feet.

It is unclear the roles that each of the rumen microbial species in the species-level profile may play in feed efficiency. More research is necessary to determine whether and how the species identified in the profile interact with the host and with one another.

IMPLICATIONS

The successful prediction of RFI status and predicted RFI value for ewe lambs in this study using a known microbial profile is a promising step toward predicting feed efficiency in ruminant livestock. The ability to predict feed efficiency without the need to measure individual feed intake, which can be a laborious and expensive undertaking, would allow producers to more easily incorporate it into their genetic selection criterion. Improved feed efficiency in the flock could translate into decreased feed costs or increased stocking rates, both of which can translate into increased profitability. Incorporating a profile of microbial species associated with feed efficiency into recent technology advancements, such as handheld DNA sequencing, may provide producers with a chute-side method that is faster and more economical for determining feed efficiency. Furthermore, narrowing the rumen microbial profile used to predict feed efficiency down to just a few species could lead to simple and inexpensive tests that can be performed alongside other practices (e.g., vaccinating or weaning) that bring the flock into the corral. Additionally, prediction of feed efficiency in young animals would provide producers the ability to make feed efficiency selection decisions earlier than is currently possible. Further research is necessary to determine whether these profiles can be used to successfully predict the feed efficiency status of other groups of Targhee ewe lambs and whether the profile can also be used to predict feed efficiency across various lifestages, sexes, breeds, and/or species of the host, and feeding regimes.

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