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Title: Exploring the role of the rumen microbiota in determining the feed efficiency of dairy cows

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Final Scientific Report - Publication Summary (numbers) (G6b)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1			1
Submitted, in review, in preparation	1			1
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses	1			1
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	2	1	2	6
Longer Visits (Sabbaticals)				

Description of Cooperation:

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed

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Abstract

Expanding world hunger calls for increasing available food resources. Ruminants have the remarkable ability to convert human-indigestible plant biomass into human-digestible food products, due to a complex microbiome residing in the rumen compartment of their upper digestive tract. One way to tackle the problem of diminishing food resources is to increase the animals' energetic efficiency, i.e., the efficiency with which they convert energy from feed, thereby increasing food availability while lowering the environmental burden, as these animals would produce more and eat less. We hypothesize that the cow's feed efficiency is dependent on the taxonomic composition, coding capacity and activity of its reticulorumen microbiota. To test this hypothesis, three aims are defined: (1) Evaluation of the feed efficiency of 146 dairy cows and defining two groups representing the highest and lowest 25% using the Israeli group's unique facility; (2) Comparing these two groups for microbiota diversity, identity and coding capacity using next-generation sequencing and metagenomic approaches; (3) Comparing the reticulorumen microbiota metabolic activity parameters. We measured feed efficiency in 146 milking cows and analyzed the taxonomic composition, gene content, microbial activity and metabolomic composition of rumen microbiomes from the 78 most extreme animals. Lower richness of microbiome gene content and taxa was tightly linked to higher feed efficiency. Microbiome genes and species accurately predicted the animals' feed-efficiency phenotype. Specific enrichment of microbes and metabolic pathways in each of these microbiome groups resulted in increasing valuable metabolites and decreasing unusable ones such as methane in efficient animals. This ecological and mechanistic understanding of the rumen microbiome could lead to an increase in available food resources and environmentally friendly livestock agriculture.

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Achievements

Construction of a rumen metagenome reference dataset

To determine whether there are microbiome features that are associated with the cow's energetic efficiency, we first determined the individual feed efficiency of 146 Holstein Friesian cows. Each animal was automatically monitored for multiple parameters used to calculate feed efficiency. For further analyses, we chose the upper and lower 25% of the animals that exhibited extreme feed efficiency values, for a total of 78 animals—40 efficient and 38 inefficient. Metagenomic DNA samples of these animals' rumen microbiomes were subjected to 16S rRNA gene sequencing and whole-genome shotgun sequencing. The metagenomics reads of all samples were pooled and assembled, and the predicted genes served as a reference dataset.

Microbiome features differ and can predict feed-efficiency phenotype

A comparison of microbiome richness across the animals revealed significantly lower richness in the efficient cows' microbiomes in both species and gene content. The differences in taxon richness were apparent up to the phylum level, further stressing the intensity of this phenomenon. The differences in richness were also accompanied by significantly lower diversity and higher dominance in the efficient animals' microbiomes at the species and gene levels. These differences were apparent up to the family level. These differences in microbiomes of efficient and inefficient cows begged the question of whether microbiome features could be used as markers for the feed-efficiency trait.

Thereupon, we used the species and gene composition of the rumen microbiomes to successfully predict the animals' feed-efficiency phenotypes with up to 95% accuracy using the k-Nearest Neighbors algorithm¹. For the feature-selection process, we used a Fisher's Exact test to measure differences in presence/absence between microbiomes of efficient and inefficient animals. The species and genes were ranked separately according to their *P*-values in ascending order and divided into bins of 100 features to be used for prediction. Each bin was tested for its ability to predict high or low feed-efficiency. The first species bin's prediction accuracy was 80%, while the first gene bin reached an accuracy of 95% (Fig. 1). This high prediction accuracy indicated that the differences in microbiome gene content and taxonomic composition could be used to classify and predict the cow's energetic efficiency.

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Microbiome metabolic activity varies in cows with different feed efficiencies

Following our findings of evident differences in these parameters, we further investigated the functionality of the rumen ecosystem. We targeted and measured the processes and products of different trophic levels of the rumen microbiome from efficient and inefficient cows, starting from degradation of the ingested plant fiber to the end products.

Significant differences were discovered in most short chain fatty acids (SCFAs). Out of the six SCFAs measured, four—propionate, butyrate, valerate and isovalerate—were at higher concentrations in the rumen of efficient cows. In addition, the total concentration of SCFAs was higher in the efficient animals. A significantly lower concentration of lactate was also apparent in the efficient animals' rumen. This, together with the higher concentrations of specific SCFAs, in particular propionate, implied the involvement of lactate-utilizing bacteria in producing the SCFAs². This finding was congruent with our measurements of the microbiomes' methanogenesis potential, where it was evident that the efficient cows' microbiomes produce significantly less methane than their inefficient counterparts. The finding of higher concentrations of SCFAs and lower methane emission from the efficient rumen microbiomes is consistent with the notion that propionate and butyrate production competes with methanogenesis for hydrogen and presents an alternative mechanism that serves as an electron sink³. The production of more SCFAs and less methane by the efficient cows' microbiomes is in agreement with the higher energetic efficiency. SCFAs are absorbed through the rumen wall to serve the energetic needs of the animals; propionate, for example, is the main precursor for gluconeogenesis in animals^{4, 5}. This is not the case with methane as the energy retained in it cannot be absorbed by the animals, and is lost to the atmosphere.

Differential abundance of rumen microbes and metabolic pathways

We conducted a permutative Wilcoxon rank-sum test in which gene and taxonomic profiles were compared between the microbiomes of efficient and inefficient animals. Overall, 18 species and 34,166 genes differentiated the microbiomes of efficient and inefficient cows; of these, 2 species and 227 genes were more abundant in efficient cows. The annotation and analysis of the differentiating genes against the KEGG database⁶ were in agreement with the metabolomic analysis. Among the KEGG

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pathways and resultant metabolites that were enriched in the inefficient cows' microbiomes were enzymes from the protein digestion and absorption category (peptidases), amino acid biosynthesis pathways and the methanogenesis pathway. Furthermore, a significantly lower number of KEGG pathways were enriched in the efficient cows' microbiomes, resulting in a significantly lower number of potential products. The phylogenetic annotations of genes that were enriched in the efficient cows' microbiomes were dominated by the rumen bacterial species *Megasphaera elsdenii*, a highly potent utilizer of lactate for the production of butyrate and propionate. This annotation did not appear in the inefficient cow microbiomes' enriched genes. Overall, the inefficient cows' microbiomes were less dominated by a specific taxon unique to that microbiome group, further supporting our hypothesis of higher dominance of specific functional groups in the microbiomes of efficient cows. This was also reinforced by the annotation of the two species that were differentially more abundant in the efficient cows' microbiomes in the 16S rRNA gene analysis. One annotation that appeared exclusively in this group was of the genus *Megasphaera*. *M. elsdenii* was also highly enriched in the efficient cows' microbiomes using a different genomic analysis, in which we aligned reads from our samples to a database of 58 sequenced rumen microbial genomes that are known to be involved in various rumen metabolic processes and were also identified in our previous analysis.

Conclusions

The variations in the cows feed efficiencies are reflected as changes in the microbiome functionality, where changes in the dominance of specific functional components affect the overall availability of ecosystem goods that are of high value to the hosting animal. A possible explanation for this phenomenon could stem from a more diverse use of resource compounds in the inefficient cows' microbiomes that are enriched in species, genes and KEGG pathways resulting in a wider array of output metabolites; this was also confirmed by significantly higher KEGG output metabolites. On the other hand, in the efficient cow's microbiome, simpler metabolic pathway networks result in increased dominance of specific functional components, which leads to higher concentrations of ecosystem goods that are relevant to the host (Fig. 2). Therefore, the efficient microbiomes are less complex but more specialized

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to support the host's energy requirements. This notion is exemplified by the finding of higher concentrations of SCFAs which are valuable to the hosting animal, and lower concentrations of methane which is of no energetic use to the hosting animal, in the efficient cow's microbiome. Such metabolic changes are usually achieved via the use of antibiotic growth promoters that increase the animal's feed-efficiency⁷.

Here we show that these metabolites are the outcome of microbiome structures that are naturally occurring and are highly correlated with, and predictive of the feed-efficiency phenotype. Therefore, these findings could be harnessed to reduce the use of antibiotic growth promoters in agriculture. A cardinal point that emerges from our findings is that the functional characteristics of a small number of species can have a large impact on community structure and ecosystem functioning. This, in turn, can change the productivity of the supraorganism—the host and its residing rumen microbiome.

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Fig. 1. Feed-efficiency prediction according to species and genes. Species (A) and genes (B) that differed in abundance between efficient and inefficient cows were ranked according to their *P*-values and grouped into bins of 100. The bins were used as predictive features for the RFI feed-efficiency parameter using the KNN algorithm with $k=3$. Each iteration used a different bin as predictive features, in ascending *P*-value order. Inset in both graphs represents the first five prediction-accuracy values (*P*-value of 0.009).

Fig. 2. Consolidated model. From left to right: identical key input metabolites are ingested by the cow and presented to either an efficient microbiome (top panel) with lower richness and diversity, or an inefficient microbiome (bottom panel) with higher richness and diversity. Differences in richness result in the production of different metabolites. The efficient microbiome produces a smaller range of output metabolites than the inefficient microbiome, however with larger amounts of relevant output metabolites which are available for the animal's energetic needs.

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Description of cooperation

The animal experiment and metabolic measurements and analysis were done in Israel and the metagenomic rumen DNA samples were sequenced in the USA as mentioned our proposal. Analysis of the results is done in a cooperative and synergistic manner as well as the writing of the papers originating from this work.

List of publications

Jami E, White BA, Mizrahi I (2014) Potential Role of the Bovine Rumen Microbiome in Modulating Milk Composition and Feed Efficiency. PLoS ONE 9(1): e85423. doi:10.1371/journal.pone.0085423.

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Published articles	Jami E, White BA, Mizrahi I (2014). Potential Role of the Bovine Rumen Microbiome in Modulating Milk Composition and Feed Efficiency. PLoS ONE.
Submitted articles	Kruger Ben Shabat S, Sasson G, Faigenboim A, Durman T, Berg Miller ME, White BA, Shterzer N, Mizrahi I (2015). Deciphering rumen microbiome mechanisms involved in energy harvest efficiency of ruminants

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