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# Differences in Fecal Bacterial Community Composition Between Beef Steers which are High-Shedders and Low-Shedders of Shiga Toxin-Producing *Escherichia coli* (STEC)

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# Differences in Fecal Bacterial Community Composition Between Beef Steers which are High-Shedders and Low-Shedders of Shiga Toxin-Producing *Escherichia coli* (STEC)

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## Summary

The community composition of the fecal microbiota was compared between beef steers which were high-shedders and low-shedders of Shiga toxin-producing *Escherichia coli*. Based on Shannon and Chao 1 diversity indices, the high-shedders had a more diverse fecal bacterial community than the low-shedding steers. Members of the genus *Prevotella* were observed as being more abundant in the low-shedders compared to the high-shedders, while *Succinivibrio* were more abundant in the high-shedders. Isolation of specific bacteria which are significantly more abundant in low-shedders may pave the way to developing direct-fed microbials which are effective in reducing STEC shedding among high-shedding beef steers.

## Introduction

Shiga toxin-producing *E. coli* (STEC) are important foodborne pathogens whose natural reservoir happens to be the gastrointestinal tract of ruminants. Of particular relevance to the beef industry is the fact that seven major STEC serogroups, namely O157, O111, O145, O45, O26, O103, and O121, are considered adulterants in beef and beef products. Therefore, intervention strategies need to be developed to minimize contamination of beef by these pathogens. A better understanding of the factors which play a part in the shedding of

STEC by beef animals is an important prerequisite to the development of such intervention strategies.

The purpose of this study was to understand the role played by the commensal gut microbiota of beef steers in relation to STEC shedding, through characterization and comparison of the fecal microbiotas of STEC high-shedders and low-shedders.

## Procedure

Fecal samples were collected from 170 beef steers during August 2011, and animals that were high-shedders and low-shedders of STEC were identified as described previously (2013 *Nebraska Beef Cattle Report*, pp. 92-93). DNA was extracted and purified from 48 of the highest shedders and 48 low-shedders using the MagMAX™ Pathogen RNA/DNA Kit

(Life Technologies Corp., Carlsbad, Calif.) according to manufacturer's instructions. The V1-V3 regions of the 16S rRNA genes from the fecal bacterial community of each fecal sample were amplified using the polymerase chain reaction (PCR) technique. The resulting amplicons were multiplexed and were subsequently sequenced at the Genome Center, University of Oklahoma, using 454 pyrosequencing ([www.454.com/](http://www.454.com/)). The resulting sequence data were analyzed using the published bioinformatic pipelines of MOTHUR ([www.mothur.org/](http://www.mothur.org/)) and QIIME ([qiime.org/](http://qiime.org/)).

## Results

The taxonomic composition of the most abundant phyla and genera in the two shedding phenotypes is compared in Figures 1 and 2, respectively.

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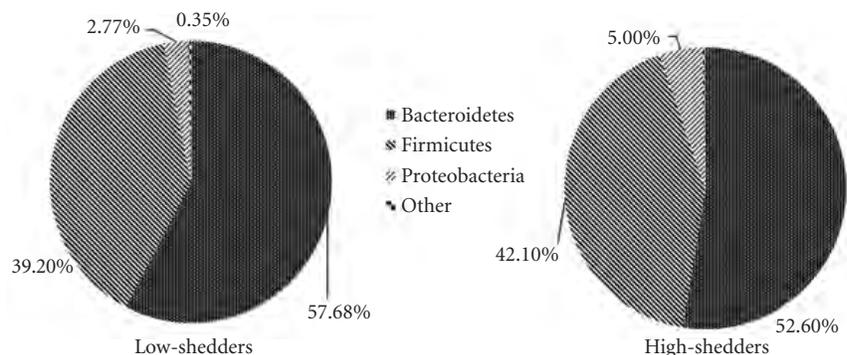


Figure 1. Phylum level taxonomic distribution of bacteria in high- and low-shedder fecal samples.

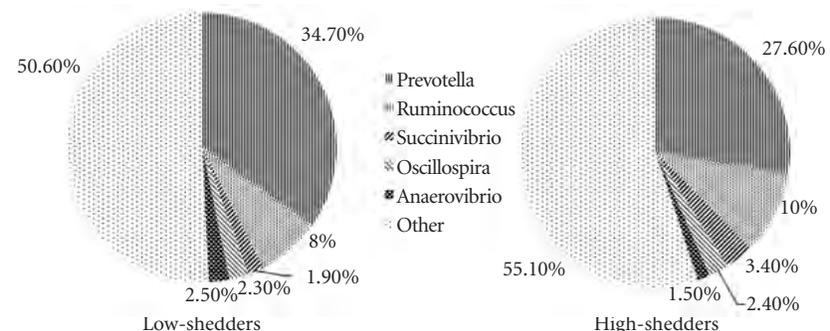


Figure 2. Genus level taxonomic distribution of bacteria in high- and low-shedder fecal samples.

The phyla Bacteroidetes and Firmicutes were the most abundantly represented phyla of both the high-shedder and low-shedder fecal bacterial communities. The phylum Proteobacteria, however, was represented more in the high-shedders. At the genus level, members of the genus *Prevotella* were more abundant in the low-shedders, while members of the genus *Succinivibrio* were more represented in the high-shedders.

The abundance of several operational taxonomic units (OTUs — which are roughly equivalent to bacterial species), were significantly different ( $P < 0.05$ ) between the two shedding phenotypes as shown in Figures 3 and 4.

The 170 animals from which the fecal samples were collected were on three different diets: a corn-based control diet (CON), DDGS, and WDGS. The distribution of these three diets among each of the two shedding phenotypes is represented in Figure 5.

The results presented above show that 45.83% of the high-shedders were on the WDGS diet while only 10.42% of the low-shedders were on the same diet. Conversely, a majority of the low-shedders were on the corn-based control diet, while the DDGS diet appeared similarly represented among both high- and low-shedding animals. This apparent impact of the diet on shedding phenotype suggests diet may also influence STEC shedding in cattle, which has been described (2010 Nebraska Beef Cattle Report, pp. 86-87), but was unclear if related to microbial community.

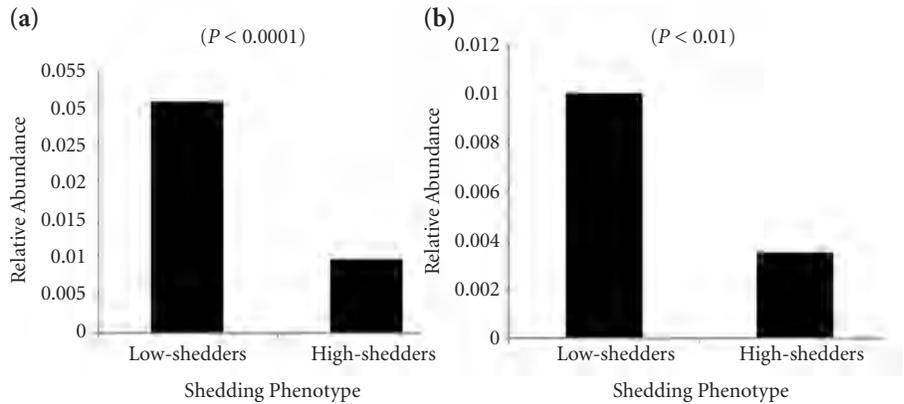


Figure 3. OTUs which were significantly more abundant in the low-shedders compared to the high-shedders. (a) OTU 13 and (b) OTU 37, both corresponding to the genus *Prevotella* (exact species unknown)

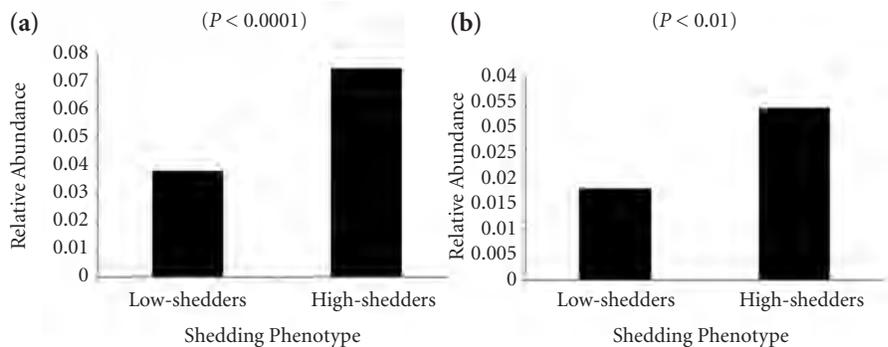


Figure 4. OTUs, which were significantly more abundant in the high-shedders compared to the low-shedders. (a) OTU 4, a member of the genus *Ruminococcus* and (b) OTU 12, a member of the *Succinivibrio* genus

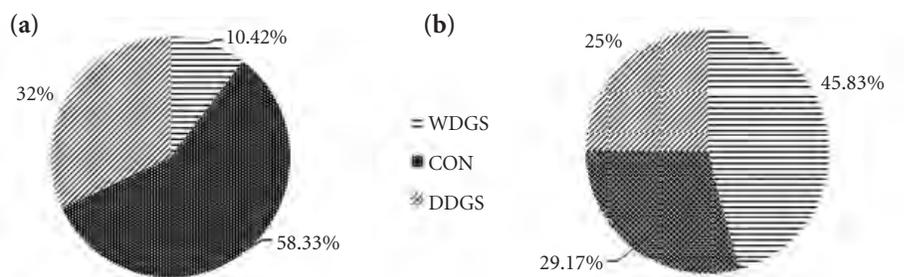


Figure 5. Distribution of the three diets among the shedding phenotypes: (a) Low-shedders (b) high-shedders

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