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Diet and feed efficiency status affect rumen microbial profiles of sheep☆

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ABSTRACT

The rumen microbiota plays a large role in the digestion of consumed feeds in ruminant livestock and likely influences feed efficiency. The objective of this study was to determine associations of diet and feed efficiency status with rumen microbial profiles in growing lambs. Growing wethers were fed either a concentrate- (C; n = 39) or forage-based (F; n = 38) diet. Individual feed intake was measured over a 49 d intake trial and initial, mid and final BW were recorded for estimation of feed efficiency. Rumen fluid samples were collected at the end of the trial, and DNA for sequencing was extracted from the rumen fluid of the 10% lowest ranking and highest ranking wethers for feed efficiency on each diet. Paired-end reads were filtered, quality trimmed and compared with a database of known 16S rDNA genes. Operational taxonomic units (OTUs) were defined as sequence clusters with ≥ 97% identity in a 16S rDNA database; 349 prokaryotic OTUs were present in at least one animal. Of these OTUs, 27 were affected ($P \leq 0.05$) by the interaction of diet with feed efficiency status, 44 were affected ($P \leq 0.05$) by the main effect of diet, and 11 were affected ($P \leq 0.05$) by the main effect of feed efficiency status. These results confirm that diet is a major influence on composition of the rumen microbiome. Also, key microbial species may play important roles in the regulation of feed efficiency, and those species may differ according to diet composition.

1. Introduction

The rumen microbiota ferments feedstuffs into fermentation end-products, especially volatile fatty acids (VFA). These compounds can then be absorbed across the gastrointestinal tract (Bergman, 1990; Russell et al., 1992; van Soest, 1994). These VFA include, in descending order of abundance, three single chain VFA: acetate, propionate, and butyrate, and three branched-chain VFA: isobutyrate, isovalerate, and valerate. These VFA are known to differ in proportion by diet. The biological pathway of nutrients associated with rumen microbes can significantly influence the maintenance, growth and performance of the host. In turn, the host provides an environment that is both anaerobic and substrate-rich in which microbes are able to thrive. This symbiotic relationship benefits both host and microbiota. Several factors can affect microbial composition in the rumen, including type and composition of feed, age and health of host, environmental temperature, and geographic location (Bryant, 1959; von Keyserlingk and Mathison, 1993). Diet is the main determinant of rumen microbial composition and VFA molar concentration among cattle and sheep (Carberry et al., 2012); however, there is a need for greater understanding of how rumen bacterial communities in livestock are associated with feed efficiency. Guan et al. (2008) first reported differences in microbial profiles generated using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) in feedlot steers that differed in feed efficiency, suggesting that microbial composition may play an important role in the efficiency of feed use. Furthermore, while microbial type and function of many individual genera and species in the rumen have been studied, the overall composition and behavior of how specific rumen microbes interact with each other and with the host related to feed efficiency is not well understood.

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One aspect of this limited understanding concerns how the rumen microbiota is implicated in methane production. The importance of methane-producing bacteria has recently come to light because methane production by ruminants has been identified as a potentially manageable environmental impact of livestock production. Approximately 19% of total worldwide methane emissions are attributed to enteric fermentation and manure (Knapp et al., 2014). Domesticated livestock alone contribute greater than 13% of global methane emissions, and ruminants can lose 5.5–10.0% of their ingested energy through methane production by way of eructated gases from the rumen during fermentation of feedstuffs (Johnson and Ward, 1996; Nicholson et al., 2007). Differences in methane output by individual ruminant animals have been linked with variation in feed efficiency measured as an animal's efficiency in converting ingested feeds into a desired output, such as growth (Carberry et al., 2012). High efficiency steers fed a barley grain-based diet were reported to produce 25% less methane than their low efficiency contemporaries (Hegarty et al., 2007). Reducing methane production in ruminants could not only contribute to lower methane emissions, but also improved feed efficiency and productivity.

Because feed costs for livestock are a substantial portion of production costs in animal agriculture, improving feed efficiency becomes more important, especially during time periods of elevated feed costs or reduced livestock values. Improvements in feed efficiency can reduce feed usage while maintaining animal performance. Establishing a relationship between feed efficiency and rumen microbial profiles could facilitate selection of more efficient breeding stock without the need to collect individual animal feed intake data, an expensive and time-intensive task. Work relating feed efficiency with rumen microbial profiles in sheep has been limited to-date, but research in this area has become more prevalent with improved, affordable sequencing technologies. Furthermore, Henderson et al. (2015) reported that the rumen microbiome is dominated by a core community of microbes regardless of ruminant species (domesticated or wild, globally), and that diet had a greater influence on the rumen microbiome than species. These data suggest that, while the majority of the studies conducted in this area have been in cattle, comparisons of rumen microbiota across host species may be warranted. The objective of this study was to determine associations of diet type and feed efficiency status with rumen microbial profiles and VFA concentrations in growing lambs. We hypothesized that microbial profiles and VFA concentrations in lambs would differ with diet and also with feed efficiency status, which would have implications for feed efficiency selection in ruminant livestock. We also hypothesized that a group of rumen microbes would differ between more and less efficient lambs, regardless of diet composition.

2. Materials and methods

2.1. Animals and diet

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. Growing wethers (n = 77; initial body weight = 51.3 ± 1.2 kg) of Rambouillet, Hampshire, and Suffolk breed types were stratified by body weight to receive either a concentrate (C; n = 39) or forage-based (F; n = 38) pelleted diet (Table 1). Diets differed in composition (CP, ADF, NDF, etc.) by design in order to determine whether there were a group of microbes that differed between high and low feed efficiency animals regardless of diet composition. Furthermore, both diets were pelleted to eliminate variation of ingested particle size, which is known to have an effect on the microbiome (van Soest, 1994). Lambs were acclimated to diets using a 20% increase in proportion of new feed to old feed every 4–5 d until the diet consisted of 100% of the new pelleted diet *ad libitum*. Individual feed intake was measured using an automated feed intake system (GrowSafe Systems Ltd.; Airdrie, Alberta, Canada) over a 49 d trial period and 2-d average initial and final body weight (BW) were recorded. Performance traits estimated over the 49 d trial period included: average daily feed intake (ADFI), average daily gain (ADG), and residual feed intake (RFI). Residual feed intake is a desirable measure of feed efficiency in livestock production because it is moderately heritable and independent of growth and mature size (Carberry et al., 2012). It is defined as the difference between actual feed intake, as recorded by the GrowSafe system, and expected feed intake. Expected feed intake was estimated by multiple regression of actual feed intake on individual ADG and metabolic midweight [body weight$^{0.75}$] (Cammack et al., 2005). By definition, a more negative RFI indicates greater feed efficiency (consumed less feed than expected), and a more positive RFI indicates lesser feed efficiency (consumed more feed than expected). Wethers were ranked on RFI (most negative to most positive RFI) within each diet, and the 10% highest ranking were deemed high feed efficiency (H-EFF; n = 4) and the 10% lowest ranking were deemed low feed efficiency (L-EFF; n = 4). Unfiltered (in order to include both solid and liquid state) rumen fluid samples (≥ 6.0 mL) were collected from all wethers in the morning on the last day of the trial, prior to measuring BW, 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. Any remaining rumen fluid was retained for VFA analysis and was stored at −20 °C until processing.

Table 1
Composition of pelleted diets.

Item	F[1]	C[2]
Ingredient, % DM[3]		
Alfalfa pellets	67.70	–
Corn	–	50.20
Wheat middlings	27.50	31.00
Corn gluten	–	10.00
Cane molasses	2.50	2.50
Salt	1.34	1.76
Calcium carbonate	0.60	2.30
Dried distillers grains with soluble	–	1.00
Calcium sulfate	–	0.75
Potassium chloride	–	0.19
Trace minerals and vitamins[4]	0.34	0.36
Analyzed nutrient composition		
DM, % as fed	92.30	91.60
CP[5], % DM	16.20	12.10
NDF[6], % DM	36.30	17.60
ADF[7], % DM	25.10	6.60
ME[8], Mcal/kg	2.31	2.98
Ca, % DM	1.20	1.30
P, % DM	0.37	0.47
IVDMD[9], % DM	63.15	81.47

[1] F = foraged-based pelleted diet.
[2] C = concentrate-based pelleted diet.
[3] DM = Dry matter content of feed.
[4] Includes Selenium 1600, Sheep TM ORG-Zn, Flavor APF-168, Vit E 20000 IU/#, and CHS/PN VT-FDLT.
[5] CP = Crude protein.
[6] NDF = Nutrient detergent fiber.
[7] ADF = Acid detergent fiber.
[8] ME = Metabolizable energy (Calculated from NRC (2007) values.).
[9] IVDMD = In vitro dry matter digestibility.

2.2. VFA analysis

Preparation of samples for VFA analysis was conducted according to Goetsch and Galyean (1983).Thawed rumen fluid samples (≥ 4 mL; pooled together from the 2 mL allocates that were remaining after DNA extraction) from the H-EFF and L-EFF wethers were centrifuged at 3000*g* for 10 min and supernatant was added to a solution containing 25% metaphosphoric acid that contained 2-ethyl butyric acid (2EB) as internal standard (2.0mg/ mL) such that the ratio of the volume of