ACCESS MICROBIOLOGY

RESEARCH ARTICLE

Khairunisa *et al.*, *Access Microbiology* 2022;4:000322

DOI 10.1099/acmi.0.000322

MICROBIOLOGY SOCIETY

OPEN ACCESS

Dominant remodelling of cattle rumen microbiome by *Schedonorus arundinaceus* (tall fescue) KY-31 carrying a fungal endophyte

Bela Haifa Khairunisa[1], Dwi Susanti[2]†, Usha Loganathan[2], Christopher D. Teutsch[3]‡, Brian T. Campbell[3]§, David Fiske[4]¶, Carol A. Wilkinson[3], Frank O. Aylward[5] and Biswarup Mukhopadhyay[2,*]

Abstract

Tall fescue KY-31 is an important primary forage for beef cattle. It carries a fungal endophyte that produces ergovaline, the main cause of tall fescue toxicosis that leads to major revenue loss for livestock producers. The MaxQ, an engineered cultivar, hosts an ergovaline nonproducing strain of the fungus and consequently is nontoxic. However, it is less attractive economically. It is not known how rumen microbiome processes these two forages towards nutrient generation and ergovaline transformation. We have analysed the rumen microbiome compositions of cattle that grazed MaxQ with an intervening KY-31 grazing period using the 16S rRNA-V4 element as an identifier and found that KY-31 remodelled the microbiome substantially, encompassing both cellulolytic and saccharolytic functions. The effect was not evident at the whole microbiome levels but was identified by analysing the sessile and planktonic fractions separately. A move from MaxQ to KY-31 lowered the Firmicutes abundance in the sessile fraction and increased it in planktonic part and caused an opposite effect for Bacteroidetes, although the total abundances of these dominant rumen organisms remained unchanged. The abundances of *Fibrobacter*, which degrades less degradable fibres, and certain cellulolytic Firmicutes such as *Pseudobutyrivibrio* and *Butyrivibrio* 2, dropped in the sessile fraction, and these losses were apparently compensated by increased occurrences of *Eubacterium* and specific *Ruminococcaceae* and *Lachnospiraceae*. A return to MaxQ restored the original Firmicutes and Bacteroidetes distributions. However, several KY-31 induced changes, such as the low abundance of *Fibrobacter* and *Butyrivibrio* two remained in place, and their substitutes maintained significant presence. The rumen microbiome was distinct from previously reported faecal microbiomes. In summary, KY-31 and MaxQ were digested in the cattle rumen with distinct consortia and the KY-31-specific features were dominant. The study also identified candidate ergovaline transforming bacteria. It highlighted the importance of analysing sessile and planktonic fractions separately.

INTRODUCTION

In the foregut or rumen of ruminants such as cattle, a complex microbial community anaerobically degrades the feed, generating volatile fatty acids as carbon and energy nutrition for the animals [1]. The process also makes livestock major emitters of methane, a potent greenhouse gas [1]. For near 100 years there has been intense research focused on rumen microbiome metabolism because

Received 06 October 2021; Accepted 13 December 2021; Published 24 February 2022

Author affiliations: [1]Genetics, Bioinformatics, and Computational Biology, Virginia Tech, Blacksburg, VA 24061, USA; [2]Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, USA; [3]Southern Piedmont Agricultural Research and Extension Center, Virginia Tech, Blackstone, VA 23824, USA; [4]Shennandoah Valley Agricultural Research and Extension Center, Virginia Tech, Raphine, VA, 24472, USA; [5]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA.

***Correspondence:** Biswarup Mukhopadhyay, biswarup@vt.edu

Keywords: cattle; pasture; grazing; rumen microbiome; remodeling; tall fescue; KY-31; MaxQ; tall fescue toxicosis; ergovaline; degradation.

Abbreviations: ANOSIM, analysis of similarities; ANOVA, analysis of variance; ASV, amplicon sequence variant; CLR, centered log-ratio; NMDS, non-metric multidimensional scaling; OTU, operational taxonomic units; PCA, principal component analysis; PERMANOVA, permutational multivariate analysis of variance; TSS, total sum scaling.

†Present address: Elanco Animal Health, Greenfield, IN, USA

‡Present address: University of Kentucky Research and Education Center, Princeton, KY, USA

§Present address: Archer Daniels Midland Company, Decatur, IL, USA.

The raw sequences of the 16S rDNA-V4 have been deposited to the NCBI SRA under BioProject PRJNA679608.

¶Deceased on November 16, 2018

Supplementary material is available with the online version of this article.

of this importance, helping not only to optimize the feed utilization efficiencies in economically important ruminants but also to develop microbial processes for bioenergy production [1, 2]. In this backdrop, a lack of detailed knowledge of rumen microbiome metabolism in cattle raised on tall fescue is a major gap as it impedes the efforts to utilize this economic grass efficiently for beef and dairy production, to assess the associated impacts on methane emission and for mining this source for novel biocatalysts.

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons. tall fescue] is one of the primary perennial cool-season forages that feeds up to 20% of beef cattle in the United States [3]. It is grown on more than 35 million acres in the transition zone of Southeastern US, known as the fescue belt [4]. In Argentina, Uruguay and Australia, this forage is grown on over 8.65, 1.24 and 2.72 million acres of the pastures, respectively [5, 6]. The most widely used variety of the grass is KY-31 and it is popular for its resilience towards pests and poor environmental conditions [4]. The resilience of KY-31 is due in part to its symbiotic interaction with a fungal endophyte, *Epichloë coenophiala* [7]. The fungus secretes a variety of ergot alkaloids that are beneficial to the grass [7]. However, one of these compounds, ergovaline, is toxic to the animals causing tall fescue toxicosis syndrome [7], which results in $2 billion of annual revenue loss for the US beef and dairy industries [8]. The use of engineered varieties of tall fescue that either carry a *E. coenophiala* strain that does not produce ergovaline (MaxQ) or are free of the endophyte (KY32, Cajun I and Bronson) have been promoted as a solution to this problem [7]. However, these varieties are less attractive economically due to higher costs for the seeds and pasture management, and KY-31 remains widely used in the fescue belt [9, 10]. The selection of tall fescue toxicosis-resistant or tolerant cattle remains an underdeveloped option [11, 12]. All these efforts do not consider the possibility that the rumen microbes instigate and could mitigate the above-mentioned toxicity, although there are indications of such possibilities. *In vitro* studies suggest that certain rumen micro-organisms transform ergovaline to lysergic acid [13] and it is thought that lysergic acid enters the blood stream of the cattle causing tall fescue toxicosis [13]. The addition of isoflavone producing grasses to a tall fescue diet reduces the severity of tall fescue toxicosis, and it is thought that isoflavones suppress ergovaline transforming microbes [8]; the mechanism of this suppression is unknown. In search of a microbial basis for the promotion and/or suppression of KY-31 toxicity, the characteristics of the faecal microbiomes of cattle raised on KY-31 and a non-toxic tall fescue have been compared, resulting in the identification of candidate micro-organisms [14, 15]. Recently, the rumen microbiome of pregnant ewes grazing tall fescue with high and moderate endophyte infection levels in parallel, were analysed [16]. However, these findings do not truly represent the events occurring in the rumen, as the faeces and rumen harbour distinct microbiomes, sharing ~30% of the species [14, 17] and in the study with the ewes [16] the rumen contents were sampled via an orogastric tube insertion, which provides preparations with underrepresentation of the fibre-attached microbial population. We hypothesized that the differences between the microbial systems that KY-31 and MaxQ enrich in cattle rumen are likely broader in nature, going beyond the transformation of ergovaline and covering the overall degradation process occurring both in the planktonic and sessile fractions. Accordingly, we have analysed the composition of the sessile and planktonic populations of the rumen microbiome of beef cattle that have alternatively grazed MaxQ and KY-31 pastures; the samples were collected through a cannula and two groups of animals with different levels of sensitivities to tall fescue toxicosis were studied. This is a rare rumen microbiome study with cattle that grazed a pasture and not fed with stockpiled hay or on a feedlot [18–20]. For small farmers in Virginia and elsewhere in the US fescue belt, grazing a pasture is the most prevalent way of raising beef cattle before the animals are moved to feed lot for finishing [21]. The results of our study have revealed novel type microbial successions and identified KY-31 as a dominant remodeler of sessile and planktonic microbial populations in the rumen, targeting both the cellulolytic and saccharolytic segments.

METHODS

Preparation of the animals

Eight Hereford-Angus cross steers (age: 18 months; initial body weight: 1079.88±31.92 pounds [$\bar{x}\pm \bar{x}\sigma$]) belonging to one of the two tall fescue toxicosis sensitivity groups, susceptible and tolerant, were used in the study. The degree of this sensitivity was assessed by the T-Snip Test (AgBotanica, LLC, Columbia, MO) that targets a proprietary set of single nucleotide polymorphisms in the genes for Dopamine Receptor (DRD2) [11] and Kell Blood Group Complex Subunit-Related Family, Member 4 (XKR4) [12], and provides a tolerance rating (0–5). The cattle with ratings of 0–1 are considered susceptible and 4–5 are most tolerant to tall fescue toxicosis. In our study, the susceptible group (n=4) had the rating of 1 and for the tolerant group (n=4) the ratings were 2–3. Each animal was fitted with a 4-inch cannula (Bar Diamond Parma, ID) at the Virginia-Maryland Veterinary Teaching Hospital (Blacksburg, VA) following the standard cannulation surgical procedure [22].

Grazing and rumen sample collection

A rotational grazing study was conducted by moving the animals between two Virginia Tech Agricultural Research and Extension Centers (ARECs) located 130 miles apart. The cattle were first allowed to graze on Jesup MaxQ [23] for 5 months at the Southern Piedmont AREC (SPAREC; Blackstone, VA) (*MaxQ-1 grazing*: 23 September 2016 – 26 October 2016), then switched to KY-31 for 23 days at the Shenandoah Valley AREC (SVAREC; Raphine, VA) (*KY-31 grazing*: 26 October 2016 – 16 November 2016), and finally returned to Jesup MaxQ (*MaxQ-2 grazing*: 16 November 2016 – 6 December 2016). At a point of each grazing period, as described in the Results, the samples of rumen contents of each animal were collected from three different locations,

top of the mat, middle of the mat, and ventral sac. For the top layer, a sample was taken directly by a gloved hand. Samples from the middle of the mat and ventral sac were collected using a custom-made sampling device consisting of a vacuum pump that was connected to a vacuum chamber. Here, each sample was drawn out through the collection tube of the sampling device and deposited directly into the respective sample container. The details of this unit are described in the Supplementary Methods and Fig. S1 (available in the online version of this article). Each sample was 500–700 ml in volume and was mixed to get homogeneity. This large volume allowed a better representation of the rumen content in a sample. The liquid and solid fractions of a sample were separated by filtration through a combination of three layers of sterile cheesecloth that had been wrapped separately and baked at 175 °C and then transported in sealed 15 ml conical Falcon tubes (catalogue number: 62406–200, VWR International, Radnor, PA) on dry ice to the laboratory and stored at −80 °C. All containers, tubes and pipes coming into direct contact of the samples were washed prior to use with Tergazyme, a detergent (Alconox, White Plains, NY), and 2% phosphoric acid to remove microbial and nucleic acid contaminations.

DNA extraction and 16S rDNA amplicon sequencing

From each rumen sample DNA was purified via bead beating and a modified version of phenol, chloroform, and isoamyl alcohol extraction, isopropanol precipitation and ethanol wash [24]; details are presented in the Supplementary Methods. To determine whether a DNA preparation contained inhibitors that could affect downstream processes, near full length 16S rRNA gene was PCR amplified using universal primers 27F and 1525R [25]. Metagenomic DNA preparations that yielded the desired amplicons were subjected to paired-end sequencing of the hypervariable region 4 (V4) of the 16S rRNA gene at the Environmental Sample Preparation and Sequencing Facility at the US Department of Energy's Argonne National Laboratory (ANL). The amplicons were generated using an optimized set of primers, 515F (Parada) – 806R (Apprill), that provides the best coverage of prokaryotic 16S rRNAs [26]. The sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA).

Quantification and statistical analysis of 16S rDNA sequences

Raw sequence data obtained from the ANL were analysed by the QIIME 2–2019.4 pipeline [27] for preprocessing and removal of contaminants. The amplicon sequence variants (ASVs) were generated by DADA2 [28] and clustered to operational taxonomic units (OTUs) using vsearch [29] at 99% sequence similarity. A pre-trained naïve Bayes classifier was used to annotate the sequences using the SILVA 132 database [30], and sequences annotated as chloroplast and mitochondria were removed from the dataset. The dataset was imported and analysed by using the R statistical packages [31].

Alpha diversity metrics were calculated using species richness estimator Chao1 of the microbiomeSeq package [32] with samples rarefied to 5598 sequences per sample (Fig. S2). Pairwise ANOVA (P<0.001) was calculated to determine the significance of the difference between the species richness of two groups. Community comparison between samples was performed using nonmetric multidimensional scaling (NMDS) analysis using Bray–Curtis dissimilarity distances, and unsupervised principal component analysis (PCA) using the data normalized into relative abundance with total sum scaling (TSS) and transformed into its logarithmic values using centred log-ratio (CLR) as described [33, 34]. Phyloseq [35] and vegan [36] were used in R to calculate sample distances (Bray–Curtis and UniFrac with average as method), perform NMDS analysis, and compute a dendrogram of the hierarchical clustering. The TSS and CLR data transformation, as well as sample ordination on PCA were performed using mixMC [33]. A non-parametric permutational analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM) of adonis function in vegan [36] were used to determine which sample parameters (genotype, rumen sample fraction, rumen depths, tall fescue variety and grazing transition) caused sample clustering (permutation: 999, P<0.05, with Bray–Curtis dissimilatory distance matrix). Relative abundance of the OTUs in various samples were estimated using phyloseq [35] and microbiome [37] packages. Core microbial community in a rumen sample was characterized from TSS normalized data at 95% prevalence threshold across all samples. The assessment of differential abundance of microbial species between two grazing periods with respect to either sample fractions (solid or liquid) or susceptibility to tall fescue toxicosis was performed using DESeq2 [38] with Wald P-value<0.001, fitType = 'parametric', and sfType = 'poscounts'. Non-parametric Kruskal–Wallis ($P_{\text{Kruskal–Wallis}}$=0.05) and Wilcoxon test with continuity correction (P_{Wilcoxon}=0.05) were performed to determine the significance differences in the relative abundance of a microbial species across rumen fraction and grazing transition.

RESULTS

Rumen samples and sequences of 16S rDNA V4 amplicons

The first set of samples were collected at the end of the initial 5-month-long MaxQ grazing period (MaxQ-1). The animals then grazed KY-31 for 24 days. At the end of this period (KY-31), a second set of samples were collected, and the animals were placed on MaxQ grazing (MaxQ-2). After 23 days of the MaxQ-2 phase, the last set of samples were collected. Under all these grazing conditions, the rumen pH remained constant (7.00±0.24); the same was the case for the animal's rectal temperature (102.7 °F±0.74; 39.3 °C±0.4) and average daily gain. Sampling in triplicate from three rumen locations, top and middle of the mat and the ventral sac, of eight animals and for three grazing periods produced 216 solid and 216 liquid fractions. Sequencing of the 16S rDNA-V4 elements of the DNA preparations generated from these samples provided 6.2 million sequences with 9403 ASVs. A clustering of

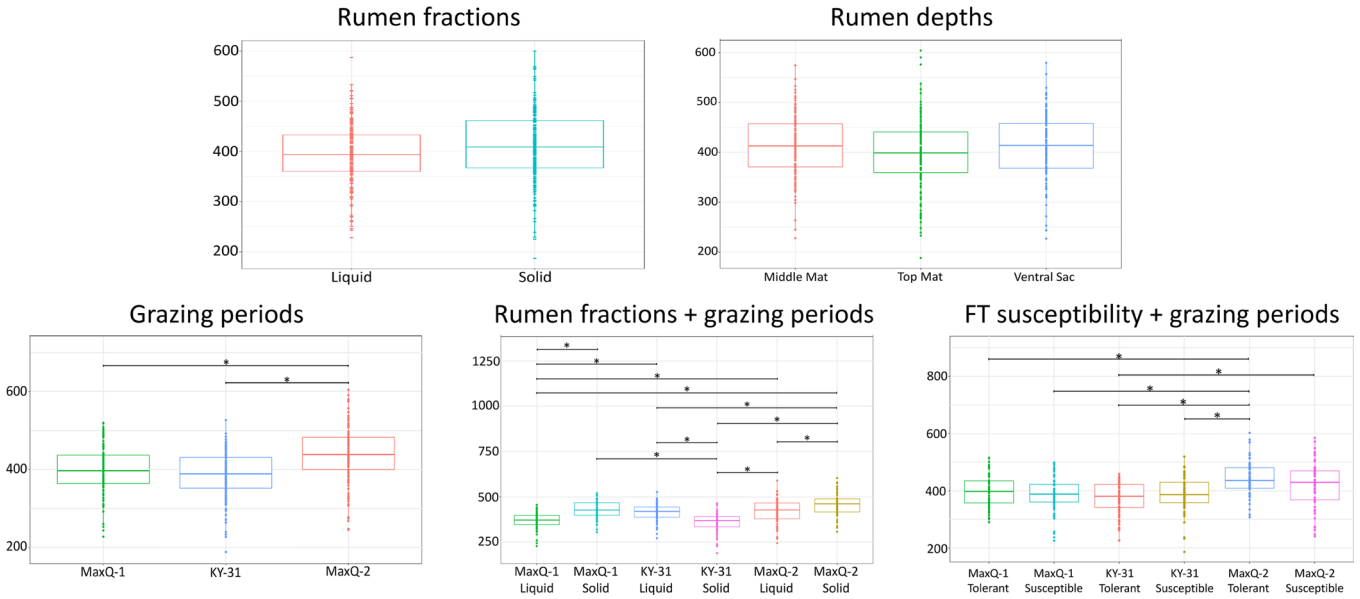


Fig. 1. Species richness of rumen microbiome of cattle grazing tall fescue. The cattle grazed the following tall fescue pastures in the sequence shown (designation of the grazing period): Jesup MaxQ (MaxQ-1); KY-31 (KY-31); Jesup MaxQ (MaxQ-2). Analysis of alpha diversity occurred across rumen fractions (solid and liquid), rumen depths, grazing periods, combination of rumen fractions and grazing periods, and combination of susceptibility to tall fescue toxicosis and grazing periods. Prior to species richness analysis, all of the samples were rarefied to 5598 reads, which was the lowest number of reads present in a single sample (Fig. S2). Horizontal bars link the samples compared in statistical analyses. *Represent P<0.001 in a pairwise ANOVA statistical test.

the data at the 99% similarity threshold produced 5547 unique OTUs. Further filtering, including the removal of 12 samples with less than 5200 reads to avoid introducing noise (Table S1), provided a final data set comprised of 5488 OTUs from 420 samples.

Alpha and beta diversity of tall fescue rumen microbiome

Overall, the species richness in the microbiome of the solid fractions of the rumen contents was similar to that of the respective liquid fraction (Fig. 1). In terms of the location within the rumen, the top of the mat harboured a less diverse microbial population than the middle of the mat and the ventral sac (Fig. 1). The grazing transitions altered the species richness significantly (Fig. 1), distinguishing the MaxQ-2 set (return from KY-31 to MaxQ) with higher species richness than MaxQ-1 and KY-31 (Fig. 1); the richness was the lowest under KY-31 grazing (Fig. 1). For both MaxQ grazing periods (MaxQ-1 and MaxQ-2), the solid fraction presented a microbiome with higher species richness than the respective liquid fraction, and the situation was opposite with KY-31 (Fig. 1). The KY-31 grazing resulted into a less diverse sessile microbial community (Fig. 1). The rumen microbiome during the MaxQ-2 grazing of cattle that were susceptible to tall fescue toxicosis had a slightly higher species richness compared to rest five sets (Fig. 1).

Community comparison via Bray–Curtis and Euclidean distances ordination (NMDS and PCA, respectively) showed clear separations between the solid (sessile) and liquid (planktonic) fractions of the microbiomes and the tall fescue types used (Fig. 2a, b). The distinctions between the rumen microbiomes on MaxQ-1, KY-31, and MaxQ-2 were more pronounced for the liquid fractions, and here MaxQ-1 samples were clearly separated (Fig. 2a, b). A similar separation, although to a lesser extent, was observed for the solid fractions. Interestingly, MaxQ-2 had some commonalities with KY-31 but not MaxQ-1 (Fig. 2a, b).

Quantitative assessment of the parameters that caused sample clustering was performed using permutational multivariate analysis of variance (PERMANOVA; 999 permutation and $P_{PERMANOVA}$=0.0001) and similarities (ANOSIM; P_{ANOSIM}=0.001) of the Bray–Curtis dissimilarity distance. Table 1 presents the respective P-values. The results show that the rumen depth was an insignificant parameter in both tests ($P_{PERMANOVA}$=0.3045 and P_{ANOSIM}=0.104), indicating that it did not contribute to the separation of the samples. In both PERMANOVA and ANOSIM analysis, the animal genotypes were found to be a significant parameter ($P_{PERMANOVA}$=0.0001 and P_{ANOSIM}=0.001) (Table 1), although it did not cause separation on the ordination plot (Fig. 3).

Tall fescue cultivar change-driven alterations in the core rumen microbiome

Covering all grazing conditions the rumen core microbiome was represented by 25 OTUs and contained bacteria with the taxonomic identities as shown in Fig. 4(b), Table S2. There were remarkable alterations in the locations of the Bacteroidetes,

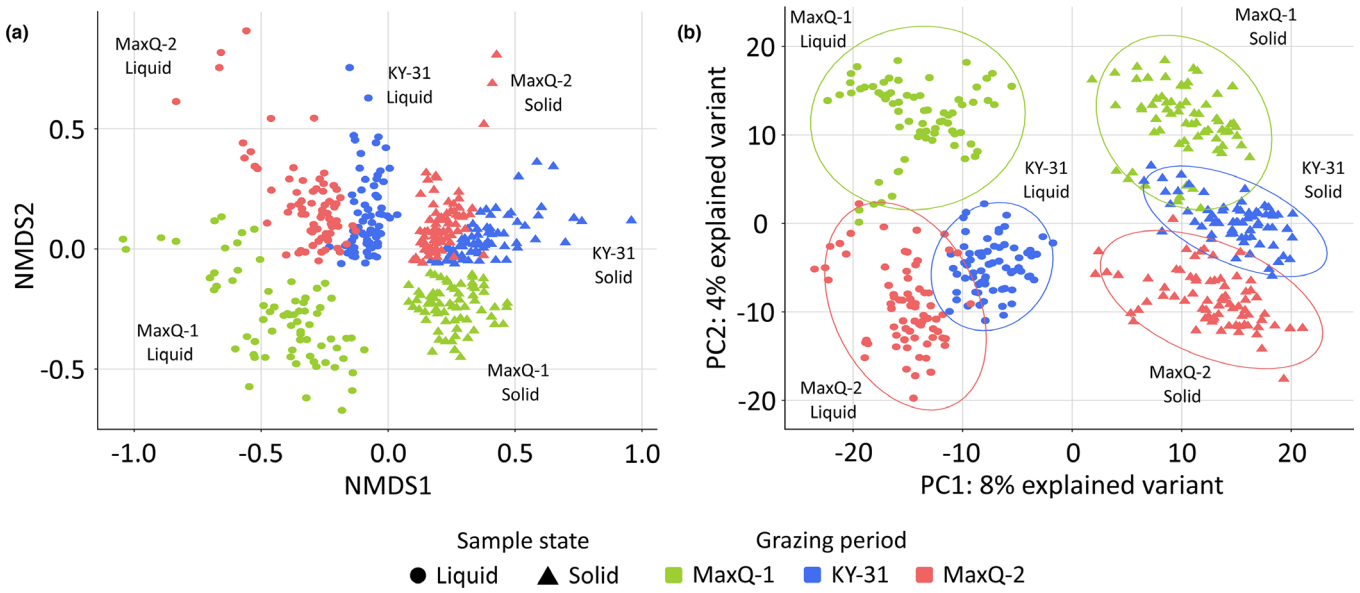


Fig. 2. Comparison of the rumen microbiome composition of cattle grazing MaxQ and KY-31 tall fescue pasture. A non-metric multidimensional scaling (NMDS) ordination of the Bray–Curtis dissimilarity distances of tall fescue rumen microbiome (a) and PCA of the TSS normalized and CLR transformed rumen microbiome data (b). The rumen microbiome was clustered based on the rumen fraction and grazing transition.

Firmicutes, Fibrobacter, and Lentisphaerae populations within the microbiome due to changes in the tall fescue type (Fig. 4a, b). We describe this effect for the sessile and planktonic populations separately. Following the transfer from MaxQ-1 to KY-31, there was a significant increase in the number of Bacteroidetes OTUs in the sessile fraction (MaxQ-1, 41.78%; KY-31, 46.44%) and then a decrease during MaxQ-2 grazing (35.33%). For Firmicutes, the observation was the opposite, as in the solid samples the abundance slightly dipped during KY-31 grazing (from 41.47% in MaxQ-1–40.38% in KY-31) before rising to 49.11% under MaxQ-2. In the liquid fraction, the abundance of Bacteroidetes OTUs continuously dropped from 41.49% in MaxQ-1–38.74 and 36.47% in KY-31 and MaxQ-2, respectively. These losses of Bacteroidetes in the liquid samples paralleled the gains in the abundance of planktonic Firmicutes (41.07, 43.84 and 46.63% for MaxQ-1, KY-31 and MaxQ-2, respectively).

Table 1. PERMANOVA and ANOSIM statistical analysis of the sample parameters

Statistical method	Parameter	*P*-value	R-value
PERMANOVA	Tall fescue variety	0.0001*	0.04835
	Genotypic factor	0.0001*	0.08395
	Rumen fraction	0.0001*	0.13672
	Rumen depth	0.3045	0.00508
	Grazing transition and rumen fraction	0.0001*	0.08488
ANOSIM	Tall fescue variety	0.001*	0.06795
	Genotypic factor	0.001*	0.1123
	Rumen fraction	0.001*	0.5885
	Rumen depth	0.104	0.003578
	Grazing transition and rumen fraction	0.001*	0.6154

PERMANOVA and ANOSIM were conducted with 999 permutations and a **P*-value below 0.05 was considered significant.
ANOSIM, Analysis of Similarities; ANOVA, Analysis of Variance; ASV, Amplicon Sequence Variant; CLR, Centered Log-Ratio; NMDS, Non-metric Multidimensional Scaling; OTU, Operational Taxonomic Units; PCA, Principal Component Analysis; PERMANOVA, Permutational Multivariate Analysis of Variance; TSS, Total Sum Scaling.

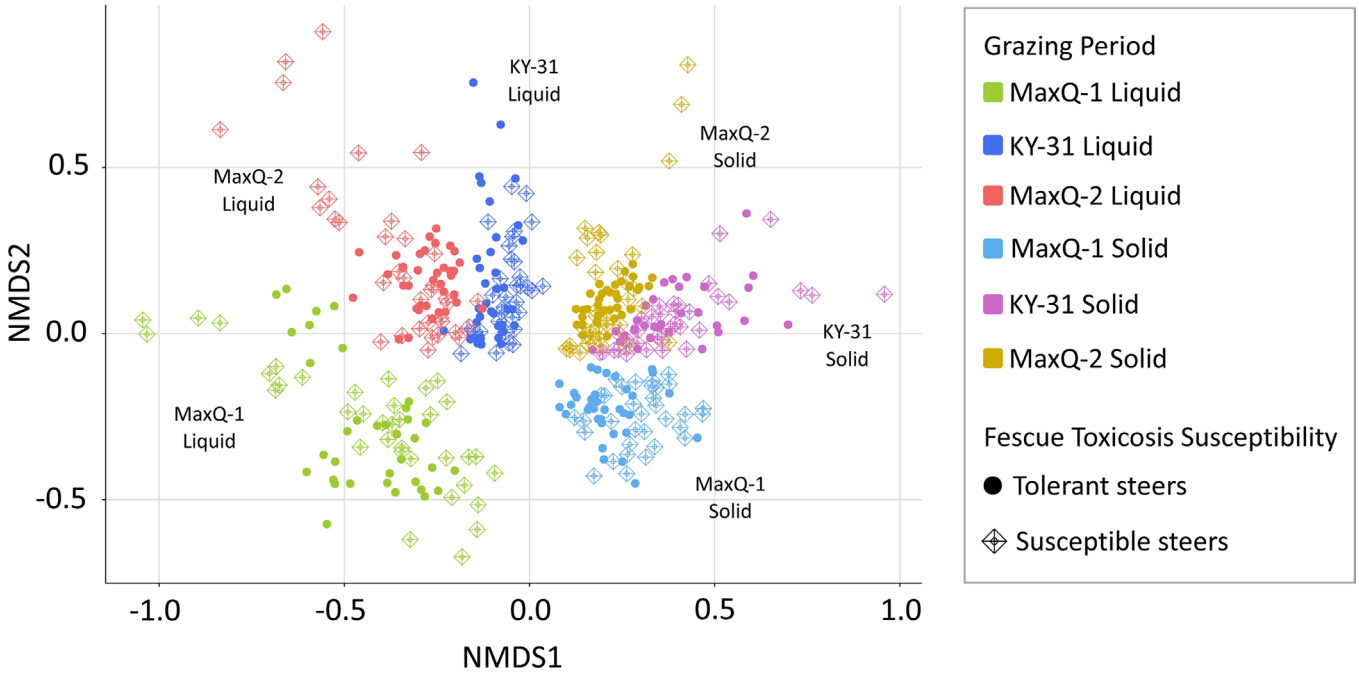


Fig. 3. Effect of cattle's genetic susceptibility to tall fescue toxicosis on their rumen microbiome compositions. The results shown are from an NMDS analysis of the data set based on Bray–Curtis dissimilarity distances.

At 95% sample prevalence, no Euryarchaea OTUs were detected in the core rumen microbiome. The prevalence thresholds that allowed the detection of the Euryarchaeota OTUs were 70, 80 and 85% for KY-31, MaxQ-1 and MaxQ-2, respectively. At 70% prevalence, the Euryarchaeota was found to be represented by the *Methanobacteriaceae* and *Methanomethylophilaceae* families under all grazing conditions. Further analysis of all the data without a prevalence cutoff showed that methanogen abundance did not change significantly with any one of the parameters studied (Fig. 5), except the KY-31 grazing slightly enhanced the abundance of species from the *Methanosarcinaceae* family in the liquid fraction (Fig. 5).

The core bacterial microbiomes of steer grazing MaxQ (pre- and post-KY-31) and KY-31 tall fescue pasture had clear differences (Fig. 4b). *Prevotella 1* genus of the phylum Bacteroidetes had higher abundance in MaxQ-1 (21%) compared to KY-31 (17%) and MaxQ-2 (16%) grazing; the % values are calculated with respect to the core microbiome OTUs. Transition to KY-31 grazing promoted the growth of *RC9* species of the *Rikenellaceae* family (Bacteroidetes phylum) significantly, as the abundance of the respective OTUs increased from 6.47 and 16.41% in the liquid and solid fractions under MaxQ-1, respectively, to 12.15 and 25.71% in the corresponding sets during KY-31 grazing. Compared to the MaxQ-1, the KY-31 grazing provided higher abundances for bacteria belonging to the candidate division vadinBE97 of the Lentisphaerae phylum, and the Firmicutes of *NK4A214* and *XPB1014* genera from the *Ruminococcaceae* and *Lachnospiraceae* families, respectively, and of unclassified Clostridiales order (Fig. 4b). Under KY-31 grazing, the population of *Fibrobacter* species of the Fibrobacteres phylum, and *Succiniclasticum*, and *Butyrivibrio 2* species of the Firmicutes and the *RF16* family of the Bacteroidales order decreased, and for the last group the drop was substantial (about 50%) (Fig. 4b. Table S2).

The transition from KY-31 back to MaxQ resulted in another reconstruction of the core microbiome characteristics. Some of the bacterial species, for which the abundance decreased during KY-31, regained their presence under MaxQ-2 to the level that was seen under MaxQ-1. This was the case for the bacteria of the RF16 family (Bacteroidales order), for which the abundance decreased from 9.74% in MaxQ-1–4.22% in KY-31 and rose to 9.41% under MaxQ-2 grazing. Similar was the observation for *Succiniclasticum* species of the Firmicutes phylum (Fig. 4b). The abundances of *Pseudobutyrivibrio* and *Butyrivibrio 2* species of the Firmicutes phyla and *Fibrobacter* remained low and did not recover in the MaxQ-2 grazing (Table S2). A non-parametric Kruskal–Wallis and Wilcoxon rank-sum test [39, 40] also showed a reduction in the abundance of Fibrobacter in sessile fraction due to shifting from MaxQ to KY-31 and a poor recovery upon return to MaxQ (Fig. 6).

Differential abundance analysis of grazing transition driven shift in microbiome

We performed a differential abundance analysis using DESeq2 [38] to identify the shifts in the microbiome composition. The use of negative binomial generalized linear model allows the comparison of log2 values for the counts for the same OTU in different

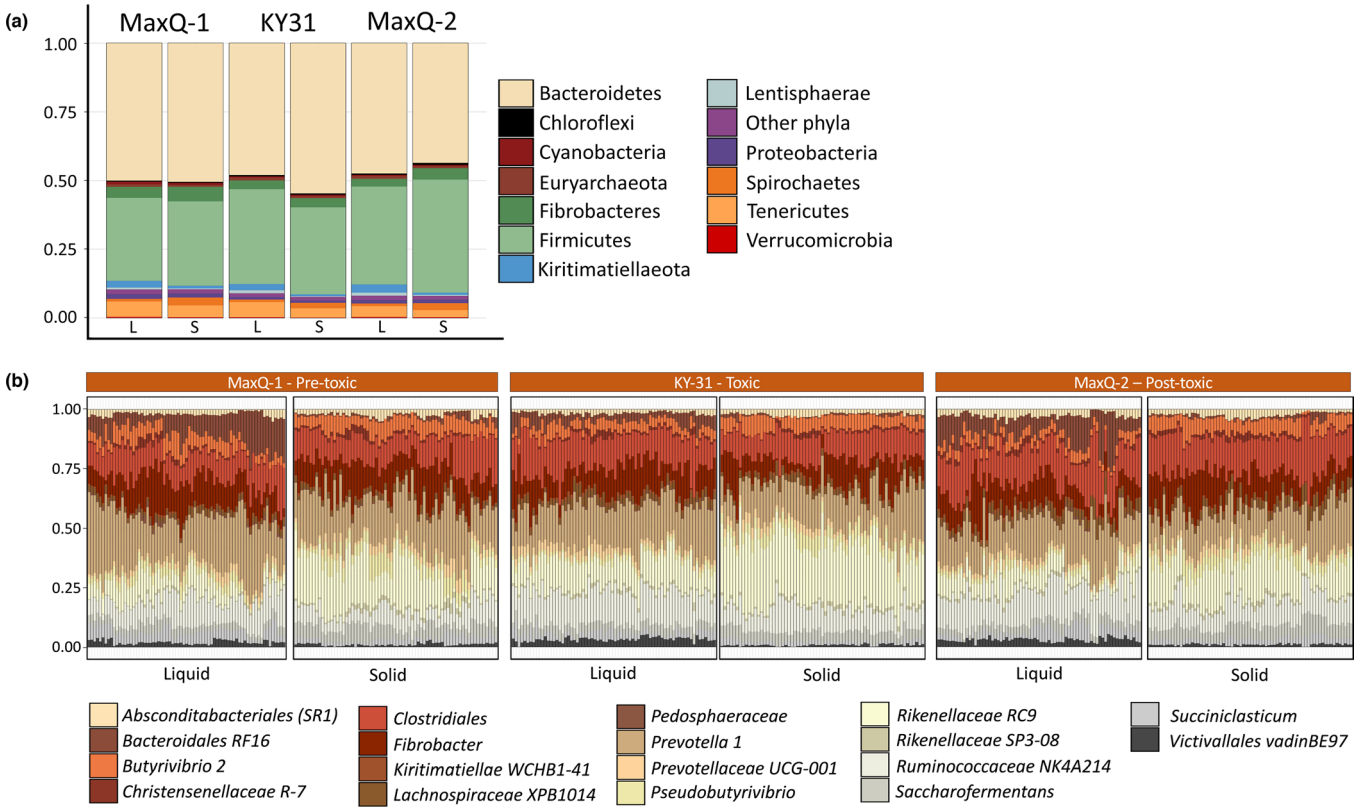


Fig. 4. Core microbiome composition shifts in the solid and liquid phases of the rumen content due to grazing transitions. Microbiome composition at the phylum-level (a) and core microbiome composition of 25 OTUs that were present at 95% prevalence threshold across all samples (b). Assigned lowest level taxonomic annotations are shown with different colours as listed at the bottom of the figure. Y-axis represents percent of relative abundance.

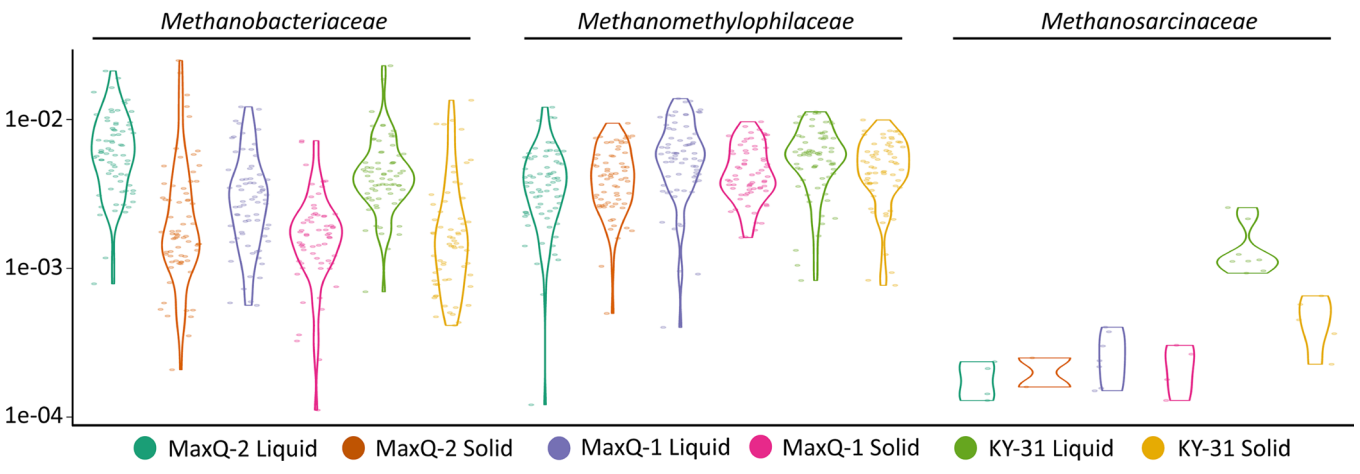
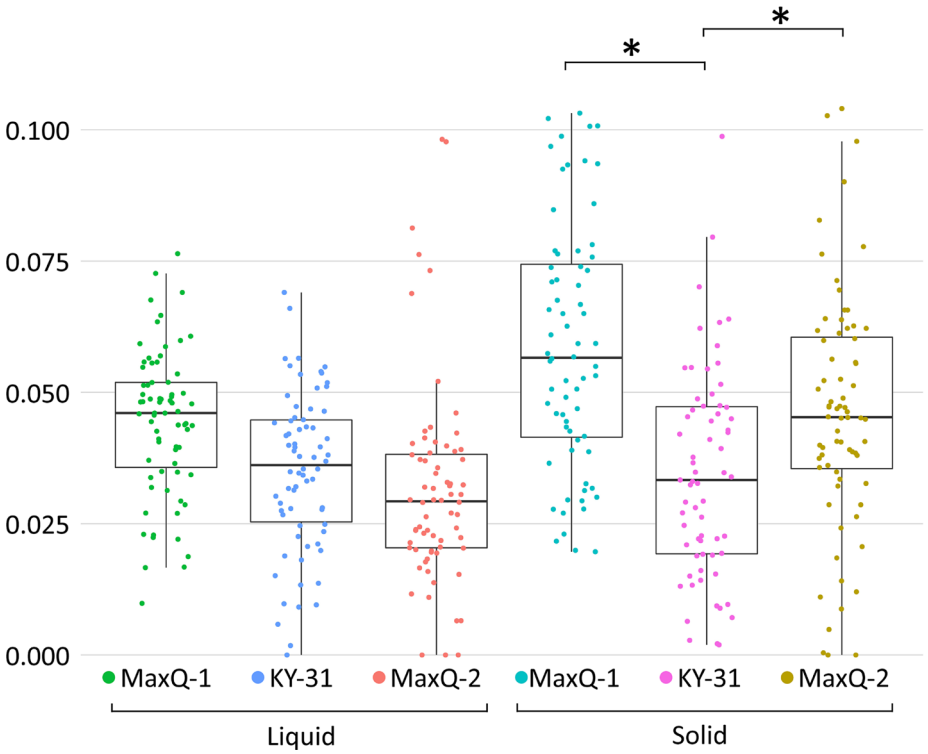


Fig. 5. Euryarchaeota abundance in tall fescue rumen microbiome. Y-axis: relative abundance of Euryarchaeota family in tall fescue rumen microbiome. X-axis: rumen fraction in each of the grazing transition are shown with different colours as indicated at the bottom of the figure. The cattle grazed the following tall fescue pastures in the sequence shown (designation of the grazing period): MaxQ (MaxQ-1); KY-31 (KY-31); MaxQ (MaxQ-2). Liquid and Solid: respective phases of rumen contents.



$P_{Kruskal-Wallis}$	
All rumen fraction	2.2×10^{-16} *
Solid	3.425×10^{-12} *
Liquid	0.0932

$P_{Wilcoxon}$ in solid fraction	KY-31	MaxQ-2
MaxQ-1	1.5×10^{-11} *	0.058
MaxQ-2	1.9×10^{-7} *	-

$P_{Wilcoxon}$ in liquid fraction	KY-31	MaxQ-2
MaxQ-1	0.96	0.14
MaxQ-2	0.15	-

Fig. 6. Relative abundance of *Fibrobacter* in the rumen microbiome during tall fescue grazing with respect to grazing periods, MaxQ-1, KY-31 and MaxQ-2, and rumen fractions. Boxplot of the relative abundance is shown on the left figure whereas *P*-values of Kruskal–Wallis ($P_{Kruskal–Wallis}$=0.05) and pairwise Wilcoxon rank sum test ($P_{Wilcoxon}$=0.05) with respect to grazing periods and rumen fraction are shown on the chart.

samples. From this analysis, enriched OTUs in each grazing comparison (MaxQ-1 vs KY-31; KY-31 vs MaxQ-2; and MaxQ-1 vs MaxQ-2) were defined as those with adjusted P_{Wald} values less than 0.001 (Table S3). A similar analysis was performed with rumen fractions as a parameter (Table S4). The results of these analyses for MaxQ-1 vs KY-31 and KY-31 vs MaxQ-2 are summarized in Fig. 7, both in terms of the total microbiomes as well as sessile and planktonic fractions, and that for MaxQ-1 vs MaxQ-2 appear in Fig. 8. The fibre-adherent community in the rumen during MaxQ-1 grazing had fourfold more abundance for *Fibrobacter* than that with KY-31, and similarly higher abundance OTUs were seen for the genus *Saccharofermentans*, family of *Lachnospiraceae AC2044,* Spirochaetes MVP-15 order, and WCHB1-41 class from Kiritimatiellae class (Fig. 7). For the Bacteroidetes phyla, a transfer from MaxQ-1 to KY-31, enriched the rumen microbiome greatly with an uncultured bacterium from the Bacteroidales order and *Rikenellaceae RC9*, *Prevotella* 1 and *Paraprevotella* species (Fig. 7). For the Firmicutes phyla, this grazing transition enriched only one species that belonged to the genus of *Lachnospiraceae FCS020*. After a month from the return to MaxQ (MaxQ-2), the abundance of a set of OTUs was elevated and these had shared annotations but not identical sequences with a group of OTUs in MaxQ-1. This observation could be interpreted as the occurrence of two groups of organisms with close but not exactly the same genetic identities in these two sets. This group was comprised of *Lachnoclostridium 10*, *Christensenellaceae R-7*, *Treponema 2*, *Anaeroplasma, Saccharofermentans* and *Lachnospiraceae AC2044* genera and uncultured bacteria belonging to the BS11 gut group of Bacteroidales order (Fig. 7).

The planktonic population in MaxQ-1 microbiome was characterized by high abundances of the following organisms: *Succiniclasticum*, *Shuttleworthia*, *Prevotellaceae UCG-003* and *Ruminococcaceae UCG-013* genera, and uncultured bacteria from *Pedosphaeraceae* family, and RF16 and BS11 families of Bacteroidales order (Fig. 7). In the liquid fraction-associated microbiome during KY-31 grazing, the abundances of *Anaeroplasma* and *Mycoplasma* species of the Tenericutes phyla, *Desulfovibrio 2* species from the Proteobacteria phyla, organisms from the Firmicutes phyla belonging to the families of *Erysipelotrichaceae* (UCG-004 and UCG-009), *Lachnospiraceae* (*Acetitomaculum* and *Lachnospiraceae* NK3A20 species), and *Ruminococcaceae* NK4A214, WCHB1-41 class of the Kiritimatiellaeota phylum, and Victivallales vadinBE97 order of Lentisphaerae phylum increased (Fig. 7). The return to MaxQ (MaxQ-2) altered the community composition again, as *Rikenellaceae RC9*, *Ruminococcaceae UCG-013*, *Ethanoligenes*, and *Tyzzerella 3* of Firmicutes phylum, *Prevotellaceae UCG-001* of the Bacteroidetes phylum, and *Pirellulaceae p-1088-a5* gut group of Planctomycetes phylum were found with significantly higher abundance here.

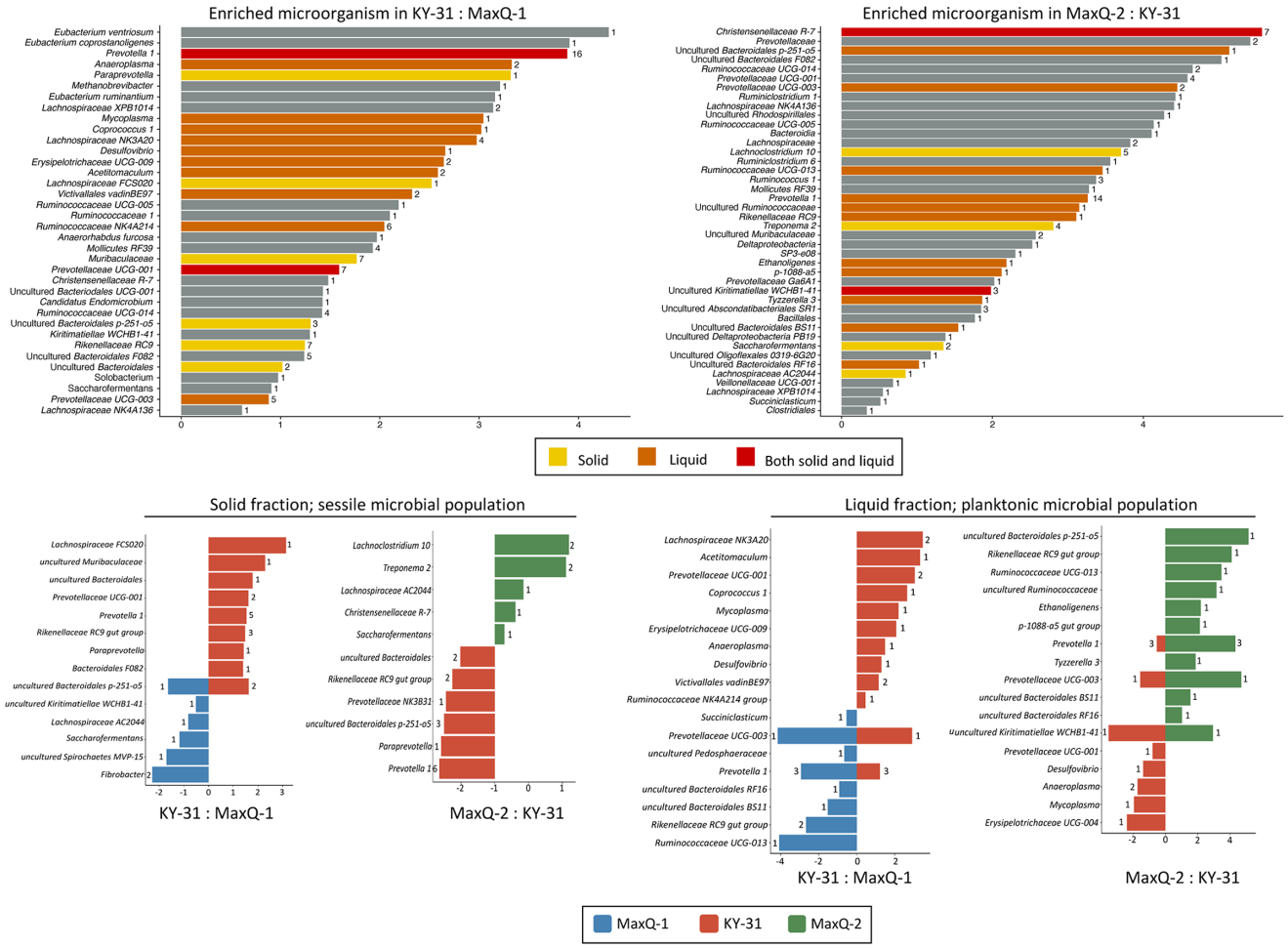


Fig. 7. Grazing transition-associated shifts in the compositions of rumen microbiome. Top plots: differential abundances of bacteria in KY-31 with respect to MaxQ-1 and in MaxQ-2 with respect to KY-31. Bottom plots: sessile and planktonic populations for the top plots. Average Log2Fold values are shown on the x-axis and the number of OTUs representing an assigned lowest taxonomic annotation is shown on the side of the respective bar.

Of the two MaxQ grazing periods, MaxQ-1 provided a higher abundance of Bacteroidetes members such as *Paraprevotella*, *Saccharofermentans*, and uncultured species of the *Muribaculaceae* family, the genera of *Blautia, Ruminococcus 1*, and *Lachnospiraceae AC2044* genera of the Firmicutes phylum, cellulolytic *Fibrobacter* from the Fibrobacteres phylum, and a *Synergistaceae* family member *Pyramidobacter* in the cattle of the rumen (Fig. 8. Bacteria of the *Treponema two* genus from the Spirochaetes phylum as well as those from the *Prevotellaceae* family, *NK3B31*, were found with a relatively exceptionally high OTU number (11 and 9, respectively) during this grazing (Fig. 8). In the MaxQ-2 grazing, *Victivallales vadinBE97* from Lentisphaera phylum, a Planctomycetes member *Pirellulaceae p-1088-a5* gut group, *Anaeroplasma* and *Mycoplasma* species from the Tenericutes phylum, *Pseudobutyrivibrio, Erysipelotrichaceae* members *UCG-009* and *UCG-004*, *Lachnospiraceae NK3A20* and *ND3007*, *Acetitomaculum* and *Tyzzerella 3* species from the Firmicutes phyla, and a *Methanobrevibacter* species of the *Methanobacteriaceae* family from Euryarchaeota phylum, were found to be enriched in this grazing (Fig. 8). Bacteria of the WCHB1-41 class from the Kiritimatiellaeota phylum, showed the highest OTU numbers (12 OTUs) while three Firmicutes genera, *Ruminococcus 2*, *Pseudobutyrivobrio*, and a known rumen acetogen *Acetitomaculum* displayed the most significant abundances (26, 40, and 70-fold higher, respectively) in the MaxQ-2 grazing (Fig. 8). An interesting pattern was seen for *Prevotella 1* species from Bacteroidetes phyla, as a total of 60 distinct OTUs for this genus were detected in the samples from MaxQ-1 and MaxQ-2 periods. While 34 *Prevotella 1* OTUs were found during MaxQ-1 grazing of which 19 were highly abundant, the corresponding values were 26 and 16 in the MaxQ-2 period, respectively. Another significant observation was that certain *Eubacterium* species, which are Firmicutes, were abundant during the KY-31 grazing irrespective of the rumen fractions (Fig. 7) and *Eubacterium ruminantium* in particular was present at a high abundance in the MaxQ-2 period (Fig. 8).

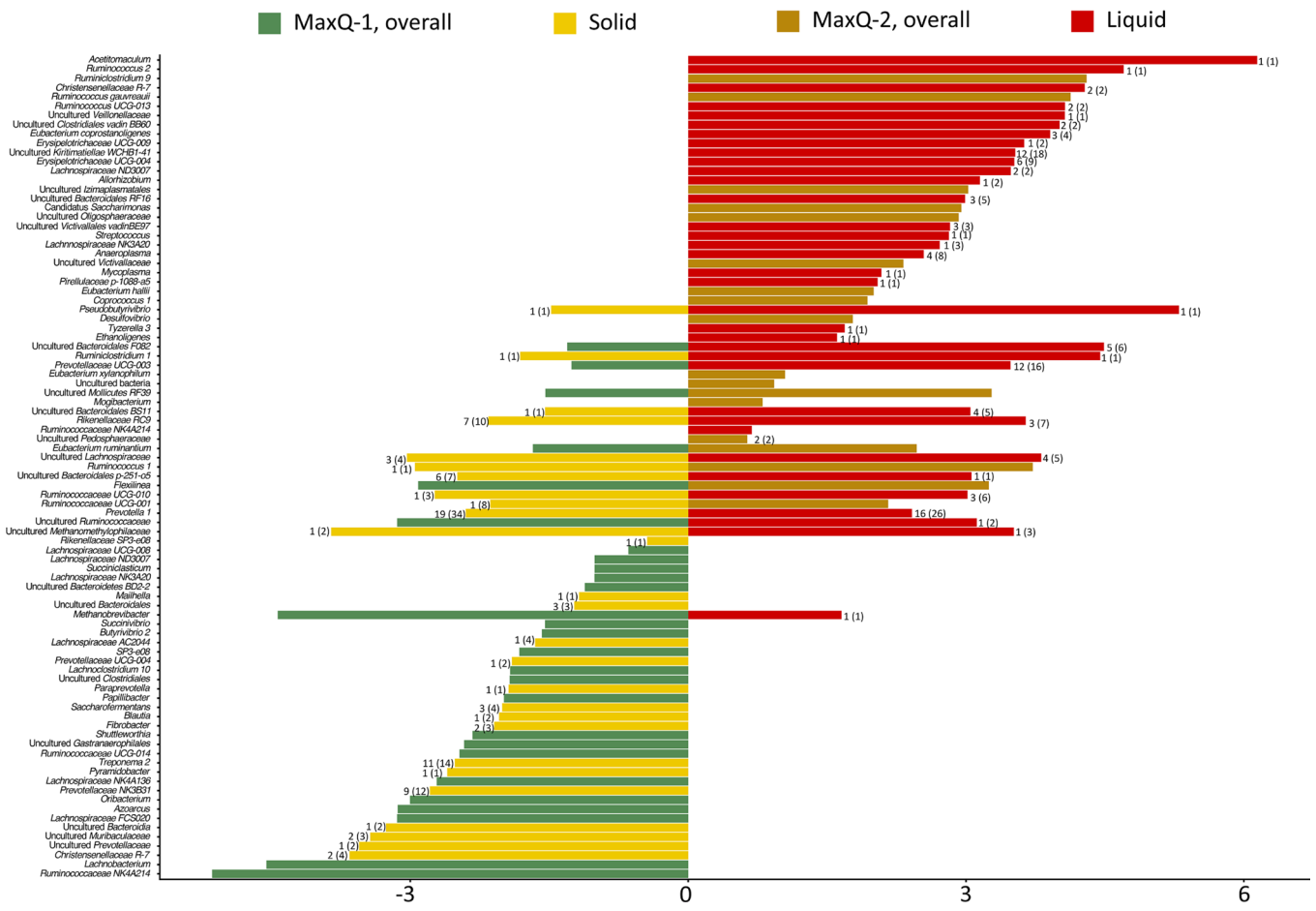


Fig. 8. Microbial community comparison between two MaxQ grazing periods. Differentially abundant bacteria in: MaxQ-1 with respect to MaxQ-2; MaxQ-2 with respect to MaxQ-1. Average Log2Fold values are shown on the X-axis. The number of OTUs representing an assigned lowest taxonomic annotation is shown on the side of the respective bar in the following format: OTUs that are significant in either solid or liquid state (total number of significant OTUs).

Host genotype-associated microbial community characteristics

A differential abundance (DESeq2) analysis showed that Bacteroidetes OTUs were significantly more abundant in the steers that are susceptible to tall fescue toxicosis (Fig. 9. Table S5), and also higher number of OTUs from the Proteobacteria phyla such as those corresponding to the *Sutterella* genus and Rhodospirillales and Deltaproteobacteria PB19 orders were also associated with this host genotype. In the rumen of this group the OTUs that had higher abundance during MaxQ-1 and MaxQ-2 grazing but were less so in the KY-31 grazing mostly belonged to *Prevotellaceae*, *Marinilabiliceae* and *Rikenellaceae* families, and some of these were annotated as unclassified Rhodospirillales order from Proteobacteria phylum and Gastranaerophilales from the newly defined phylum of Melainabacteria that is closed-related to Cyanobacteria. The cellulolytic *Fibrobacter* species were 22- and 59-fold more abundant in susceptible steers during MaxQ-1 and MaxQ-2 grazing, respectively (Fig. 9), and such a distinction was not seen during KY-31 grazing, where the abundances of these organisms were generally low.

The rumen microbiome of steers that are susceptible to tall fescue toxicosis was associated with a higher abundance of Firmicutes. Particularly, the species belonging to the R-7 group genera from the *Christensenellaceae* family showed a preferential association with the rumen of susceptible steers, being 2-, 59- and 32-fold more abundant during MaxQ-1, KY-31, and MaxQ-2 grazing, respectively, in this set compared to the toxic fescue tolerant animals (Fig. 9). In addition to elevated Firmicutes abundance, a higher association of certain members of the Tenericutes family, specifically the Mollicutes order (Mollicutes RF39, *Mycoplasma* and Mollicutes NED5E9) was observed for the rumen of susceptible steers.

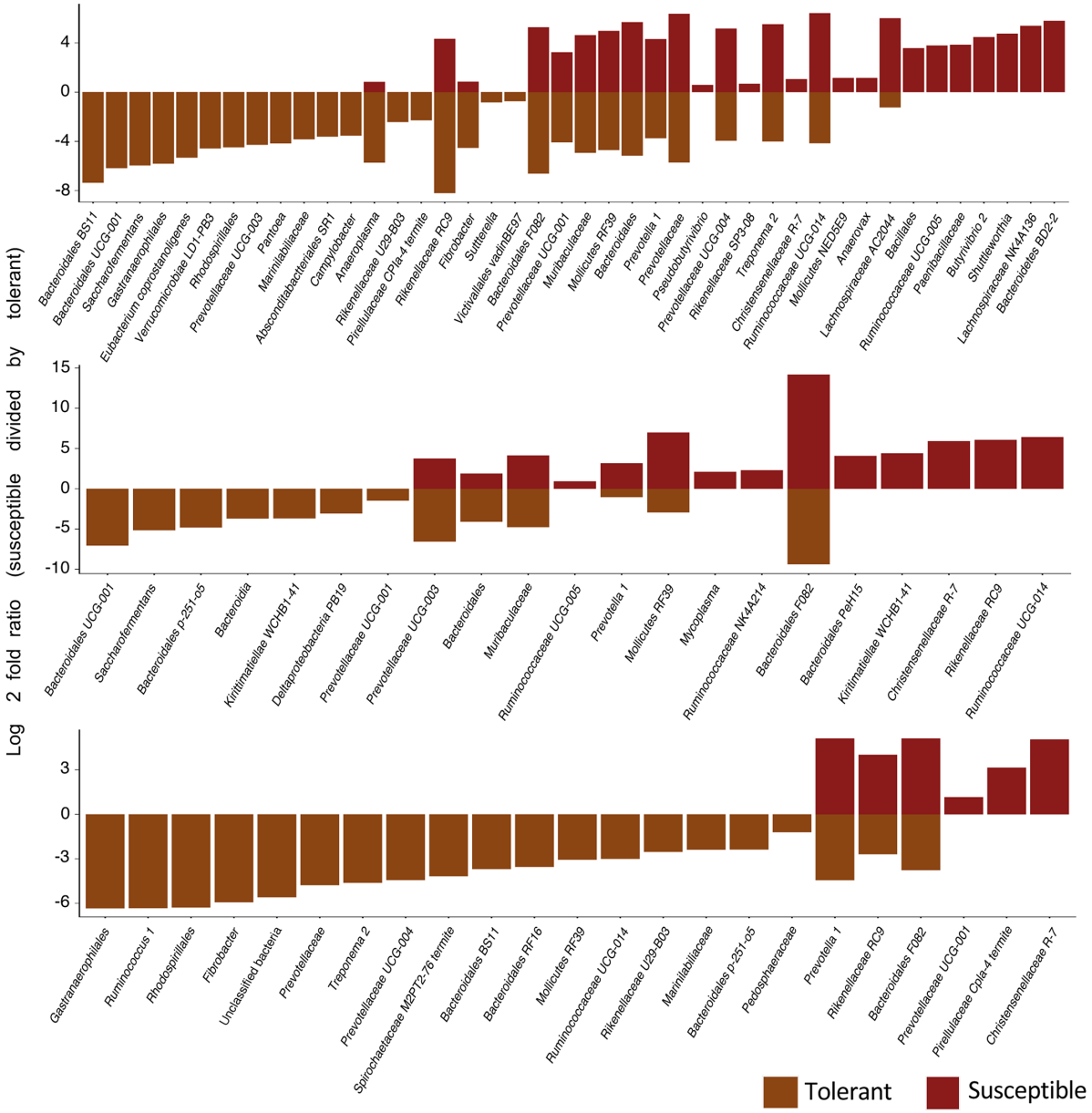


Fig. 9. Archaea and bacteria apparently enriching in the rumen microbiome based on the sensitivity of the cattle to tall fescue toxicosis. Y-axis: OTUs differentially abundant in the rumen of cattle susceptible to tall fescue toxicosis with respect to tolerant animals under three grazing conditions. The findings are expressed in terms of average Log2 fold values. Lowest attainable taxonomic annotations for the OTUs are shown at the bottom.

DISCUSSION

We have determined the differences between the compositions of rumen microbiomes of two groups of cattle that grazed a toxic and a non-toxic cultivar of tall fescue, namely KY-31 and MaxQ and inferred the respective metabolic implications. Previous studies on bovine rumen microbiome under similar nutritional conditions focused primarily on the microbial degradation of ergovaline, which makes KY-31 toxic [8, 14–16, 41, 42] and some of these employed faecal microbiome as a proxy [14, 15, 42]. We used the grazing on a KY-31 pasture as a perturbation in the middle of MaxQ grazing, and recorded the resultant changes in the rumen microbiome. This effort had two major outcomes. It showed that an analysis targeting the sessile and planktonic components separately uncovered more changes in the microbiome than the one focused on overall microbiome. Thus, an effective study of rumen microbiome cannot rely on the analysis of rumen fluid or whole microbiome only. The other was the finding that KY-31 was a dominant remodeler of the microbiome composition. A return from KY-31 to MaxQ grazing did not restore some of the original MaxQ-type features. One of these cases was a severe reduction in the

abundances of the OTUs assigned to certain key rumen cellulolytic bacteria. The location within the microbiome, sessile or planktonic segment, where Firmicutes and Bacteroidetes were found in high abundances, changed when cattle were moved from MaxQ to KY-31 grazing, and the original status in most part was restored upon return to MaxQ. *As elaborated at the end of the Discussion,* these remodelling events likely represent alterations in the microbial team composition and/or task allocations while leaving a broader ruminal metabolic function unaltered. The observation that some of the KY-31-instituted changes lingered even after a switch to MaxQ grazing represented a major departure from what has been seen with various perturbations in ruminal conditions. For example, for bloat or acidosis, the microbiome fully regains the original characteristics within 7 days of the termination of the disturbance [43, 44]. The KY-31 grazing caused an enrichment of bacteria with potential capabilities of degrading ergot alkaloids and apparently lowered the methane production potential of the rumen. The cattle's relative susceptibility to tall fescue toxicosis influenced rumen microbiome composition minimally. These observations were significant, as the 3 week long intervening KY-31 grazing, a terminal MaxQ grazing of similar duration, and much longer first MaxQ period offered sufficient times for establishing stable microbiome compositions; in cattle the sign of tall fescue toxicosis appears in 5–15 days of consuming toxic tall fescue [4] and the faecal bacterial community composition alters within 14–28 days of a shift from a mixed ration to MaxQ or KY-31 [14]. Also, the events identified in this study were distinct from those revealed with faecal microbiome as a proxy [14, 15].

At the phylum-level, the overall rumen microbiome of beef cattle grazing tall fescue MaxQ or KY-31 was most similar to those developed with Alfalfa than other diets (Table S6) [43, 45–51]. However, tall fescue provided 6–10 times more Tenericutes abundance and significantly lower level of Actinobacteria than Alfalfa (Table S6) [45]. The MaxQ grazing, provided up to twofold higher abundance of Proteobacteria compared to our KY-31 set and all above-mentioned cases [43, 45–50] (Table S6). The rumen fractions (solid and liquid) and the grazing periods (MaxQ-1, KY-31, and MaxQ-2), but not the rumen depths influenced sample clustering (Fig. 2, Table 1). The tall fescue toxicosis susceptibility of the animals was significantly correlated with only one effect (Fig. 3): the rumen of tolerant cattle contained slightly higher abundances of Bacteroidetes and Fibrobacteres while that of susceptible cattle carried a bit more Firmicutes, especially *Christensenellaceae* family members, and Tenericutes (Fig. 9).

Our analyses focused on the rumen fractions identified a potentially major microbiome remodelling event. The MaxQ-1 to KY-31 shift reduced the species diversity of the fibre-attached population and increased that of the liquid-associated population, and MaxQ-1 and MaxQ-2 grazing offered an opposite scenario (Fig. 1). A phylum level observation was even more dramatic (Fig. 4a). A move from MaxQ to KY-31 grazing reduced the relative abundance of Firmicutes and elevated that of Bacteroidetes in the sessile fraction and the opposite change occurred in the planktonic fraction, and a return to MaxQ brought back the original composition, providing a higher abundance of Firmicutes and a lowered level for Bacteroidetes in the sessile fraction and the reverse for the planktonic fraction. These effects were not seen at the total microbiome level as both KY-31 to MaxQ provided similar overall abundances of Firmicutes and Bacteroidetes in the rumen, which in combination constituted >80% of the bacterial population. However, the reversibility did not hold for certain features that are key to an optimal functioning of the rumen.

For the sessile fraction of the rumen microbiome, the transfer of cattle from MaxQ to KY-31 grazing caused an increase in the abundance of the Bacteroidetes, such as saccharolytic *Prevotella 1* and *Paraprevotella* species and RC9 of the *Rikenellaceae* family, and lowered the abundance of the Firmicutes representing major rumen cellulolytic populations with members such as *Pseudobutyrivibrio* and *Butyrivibrio* 2 (Figs 4b and 7). The opposite was the case for the planktonic population. There was also a decrease in the abundance of fibrolytic *Fibrobacter* species in the solid fraction (Fig. 6). This void in the sessile population was filled with an enrichment of cellulolytic firmicutes belonging to the *Ruminococcaceae* and *Lachnospiraceae* families (Fig. 4b, Fig. 7), and the Eubacterium genus (*E. coprostanoligenes*, *E. ventriosum* and *E. ruminantium*). The abundance of *E. ruminantium*, a typical rumen cellulolytic bacterium [52], was elevated substantially during KY-31 grazing and remained so in MaxQ-2. Bloat and acidosis are known to affect *Fibrobacter succinogenes* and the Firmicutes species such as *Ruminococcus albus* and *Ruminococcus flavefaciens* [43, 45, 53]. These conditions are associated with pH values of 5.0–5.6 [43, 54], whereas under our grazing conditions, the rumen pH remained neutral (7.00±0.24). Thus, certain KY-31-associated factor(s) were possibly deleterious to *Fibrobacter, Pseudobutyrivibrio* and *Butyrivibrio*, and their loss was compensated by the increased abundances of select Firmicutes.

For the planktonic segment of the microbiome, KY-31 grazing not only altered the level of the cellulolytic and non-cellulolytic carbohydrate hydrolysing bacterial population but also brought novel possibilities for organic and cellular material degradation activities. For example, the increased abundance of planktonic Firmicutes likely not only compensated for the above-mentioned loss of the cellulolytic population in the sessile fraction, but also enhanced the ability to degrade plant-derived organics. A member of this phylum, a *Coprococcus* species, was present at an enhanced level during KY-31, and these organisms degrade plant toxins such as nitropropionic acid, nitropropanol [55] and phloroglucinol [56]. Similarly, the *Anaeroplasma* and *Mycoplasma* species as well as the bacteria from the RF9 family of the Mollicutes order, which belong to the Tenericutes phylum and were present in elevated abundances during the KY-31 grazing, would have provided two benefits. First, their saccharolytic and proteolytic activities likely compensated for the lowered Bacteroidetes abundance in the planktonic community. Second,

their bacteriolytic and proteolytic activities were potentially useful in recycling dead cells [57]. In this context, it is noted that not all rumen mycoplasmas are bacteriolytic [57]. Proliferation of acetogens such as *Acetitomaculum*, sulfate-reducing *Desulfovibrio* species, and propionate-producing bacteria of the *Rikenellaceae* and *Erysipelotrichaceae* families indicated that under KY-31 grazing, the contribution of methanogenesis as a hydrogen sink might have been lowered [58]. This suggestion is consistent with the observation that during KY-31 grazing the rumen carried *Methanosarcina*, which can survive without hydrogen [59, 60], in a higher abundance than that with MaxQ-1 and MaxQ-2 (Fig. 5). Also, since the methanogen abundance did not change much, KY-31 or its degradation products were not likely toxic to these archaea.

The transition of the cattle from KY-31 to MaxQ provided an unexpected outcome. Even after 23 days of MaxQ-2 grazing following the KY-31 period, the rumen microbiome did not return completely to the MaxQ-1 stage (Figs 2 and 8), and exhibited some of the KY-31 stage characteristics (Figs 2 and 4). The core ruminal microbiome during the MaxQ-2 had the highest abundance of Firmicutes, comprising up to 50% of the total sequence, in both solid and liquid fractions (Fig. 4a, Table S2). One of the special features of this period was the higher abundance of Spirochaetes, specifically the species of the *Treponema 2* genus, and organisms belonging to the *Pedosphaeraceae* family of Verrucomicrobia phylum (Fig. 4). Several organisms such as the Firmicutes of the *Tyzzerella*, *Ethanoligenes* and *Succiniclasticum* genera as well as those belonging to *Bacteroidales RF16* order and *Rikenellaceae* RC9 families of the Bacteroidetes phyla that were present in the MaxQ-1 rumen microbiome in high abundance and were affected due to KY-31 grazing were able to re-establish substantial presence in the rumen under MaxQ-2 grazing. However, the abundances of *Fibrobacter* and *Butyrivibrio* 2 species in the sessile fraction, which were severely reduced in the KY-31 period, did not return to the MaxQ-1 type level during MaxQ-2 grazing (Fig. 8). On the other hand, the *Treponema* species, which were present at high levels during the MaxQ-1 grazing and had a reduced presence at the KY-31 phase gained significant abundance in the sessile fraction with the MaxQ-2 (Fig. 7). This is an intriguing observation as *Treponema* species are known to improve the cellulolytic function of cellulose degraders, especially *Fibrobacter* [61, 62]. It is likely that *Treponema* assisted *Fibrobacter* species during MaxQ-1 grazing and other cellulytic bacteria at the MaxQ-2 stage.

We have developed two hypotheses for the factors underlying the KY-31-instigated changes in the cattle rumen microbiome. The first concerns ergot alkaloids released from the grass, which could have been toxic to the fibre-adhered bacteria and served as high-value nutrients for the liquid fraction microorganisms. The fescue-associated alkaloids are known to inhibit ruminal fibre digestion [63–65]. The second hypothesis is that the distinct composition of KY-31 drove the remodelling. However, the factors causing these changes to linger remain hard to rationalize.

A comparison of our observations with those of others [14, 15, 42] suggested that faecal microbes are unlikely biomarkers for tall fescue toxicosis. In two studies where cattle were moved from a mixed ration to MaxQ and KY-31 in parallel [14, 15] or placed on KY-31 only [42], *Coprococcus one* and several other Firmicutes belonging to the *Ruminococcaceae* and *Lachnospiraceae* families, *Paraprevotella* species from Bacteroidetes phylum, and Tenericutes of *Mycoplasmataceae* family were found in higher abundances in the faeces of KY-31 tall fescue-fed cattle [14, 15, 42]. Similar changes were observed in our MaxQ-1 to KY-31 shift (Fig. 7). The faecal microbiome of cattle grazing tall fescue also contains Proteobacteria, Euryarchaeota and Lentisphaera phyla at abundances similar to those observed by us for the rumen [14, 15, 42]. However, unlike the situation with the rumen microbiome studied here, in a faecal microbiome Actinobacteria and Firmicutes are found in significantly higher abundances, while Bacteroidetes, Fibrobacteres and Tenericutes are underrepresented [14, 15, 42]. Similar is the case with alfalfa [45] and a mix of bermudagrass and Dallisgrass [50] (Table S6). Such differences are expected as the foregut deals with heterogeneous plant materials and in the hindgut undigested plant fibres are degraded [50, 66–68]. Also, the hindgut selects for microbes that can tolerate bile salts [67], which may inhibit many Gram-negative bacteria such as Bacteroidetes and Fibrobacteres and help to enrich Gram-positive bacteria such as Actinobacteria and Firmicutes [67]. In Holstein steer, the foregut and hindgut microbiomes share only 30% of the species [14, 17].

An *in vitro* enrichment study identified certain *Clostridiaceae* and *Prevotellaceae* as potential ergovaline degraders [41], and a comparison of faecal microbiomes of cattle grazing toxic and nontoxic tall fescue led to similar assigments for certain *Paraprevotella* and *Coprococcus 1* species and several members of the *Ruminococcaceae*, *Lachnospiraceae* and *Mycoplasmataceae* families [14, 15, 42]. The planktonic segment of the rumen microbiome of pregnant ewes is thought to carry ergovaline degrading bacteria from the Lachnospiraceae and Veillonellaceae families of the Firmicutes phylum and Coriobacteriaceae family of Actinobacteria phylum [16]. From the observed enrichment in the rumen as the cattle were shifted from MaxQ-1 to KY-31 grazing, we have identified *Coprococcus 1, Paraprevotella*, *Prevotella 1*, *RC9*, *Mycoplasma*, *Anaeroplasma* and several members of the *Ruminococcaceae* and *Lachnospiraceae* families as potential ergovaline degraders. These similarities between the hypotheses arising from rumen and faecal microbiome studies (current report and [14–16, 42]) suggest that ergovaline is metabolized in the rumen and large-intestine through similar processes. Ergovaline generated from KY-31 in the rumen is known to be available in the cattle's large intestine [13, 69].

CONCLUSION

The compositions of rumen microbial communities in the cattle that grazed MaxQ and KY-31 tall fescue pastures, were distinct. Interestingly, this distinction was captured only when the comparison was performed at the sessile and planktonic levels separately and not at the whole microbiome level. The MaxQ grazing helped to enrich a cellulose metabolizing community made up of *Fibrobacter*, *Pseudobutyrivibrio* and *Butyrivibrio* 2, and under KY-31 grazing the same overall function was provided by a consortium of *Eubacterium* species and certain members of *Ruminococcaceae* and *Lachnospiraceae* families. A similar change occurred in the saccharolytic team. There was an apparent enhanced need for Tenericutes for the digestion of KY-31. A major observation was that some of the effects of KY-31 grazing, such as the reduction of *Fibrobacter* and enrichment of substitute cellulolytic bacteria, lingered even after the cattle were moved to MaxQ grazing. It was hypothesized that the alkaloids produced by the fungal endophyte of KY-31 as well as the distinct compositions of MaxQ and KY-31 were the drivers for these effects. Often, beef cattle are raised by grazing tall fescue until the age of 6–12 months, and then, are placed on feedlots until the age of 18–21 months for finishing [21]. Our results suggest that the cattle grazing KY-31 and MaxQ arrive on feedlots with distinct microbiomes, and the KY-31-specific characteristics linger. Thus, a fuller understanding of the metabolic details of the MaxQ- and KY-31-specific microbiomes will help to develop strategies for mitigating tall fescue toxicosis as well as improving the feed utilization efficiencies at both the grazing and finishing steps. It will also reveal new biomass degradation systems of applied value. The next steps for obtaining such details would be the generation and analysis of metagenome assembled genomes (MAGs), transcript, protein and metabolite level analysis, and metabolic modelling.

Funding information
This work was supported with a Capacity Building grant from the Virginia Research and Extension Innovation Initiative and Virginia Cooperative Extension at Virginia Tech and a College of Agriculture and Life Sciences Pratt Endowment grant to BM, BC and CDT. BM was supported in part by the Agricultural Experiment Station Hatch Program (CRIS project VA-160151) at Virginia Tech. BHK received a Pratt Animal Nutrition Fellowship for Graduate studies from the Virginia Tech College of Agriculture and Life Sciences Pratt Endowment and a Graduate Fellowsip from the Genetics, Bioinformatics, and Computational Biology Ph.D. Program of the Virginia Tech.

Acknowledgements
We thank Danny Eanes for expert help in the construction of the sampling device, Sierra R. Guynn and Terry Swecker for the cannulation of the steers, Brad Ellis, Lauren Rush and Endang Purwantini for assistance in sample collection, and Brian Hedlund and Shrikant Bhute for advice of obtaining 16SrRNA-V4 sequencing and introducing BHK to QIIME.

Author contributions
D.S., B.H.K., C.T., D.F., C.A.W., B.T.C. and B.M. conceptualized the study; B.H.K., D.S., C.T., U.L., C.A.W. and B.M. performed the research; B.T.C., C.T., C.A.W. and B.M. received the funding. B.H.K., F.O.A. and B.M. analysed the data; B.H.K. and B.M. prepared the visualization; B.H.K. and B.M. wrote the paper. B.H.K., D.S., F.O.A., B.T.C., C.A.W. and B.M. reviewed and edited the final manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
All of the procedures with the animals were conducted following protocols that were approved by the Virginia Tech Institutional Animal Care and Use Committee (protocol #IACUC 15-065 and 17-171).

References

1. Russell JB. *Rumen Microbiology and Its Role in Ruminant Nutrition*. Department of Microbiology, Cornell University, 2002.
2. Weimer PJ, Russell JB, Muck RE. Lessons from the cow: what the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass. *Bioresour Technol* 2009;100:5323–5331.
3. Hall SL, McCulley RL, Barney RJ, Phillips TD. Does fungal endophyte infection improve tall fescue's growth response to fire and water limitation? *PLoS One* 2014;9:e86904.
4. Haschek WM, Voss KA. Chapter 39 - Mycotoxin. In: Haschek WM, Rousseaux CG and Wallig MA (eds). *Haschek and Rousseaux's Handbook of Toxicologic Pathology. II*, 3rd ed. Cambridge, Massachusetts: Academic Press; 2013. pp. 1187–1258.
5. Hume DE, Sewell JC. Agronomic advantages conferred by endophyte infection of perennial ryegrass (Lolium perenne L.) and tall fescue (Festuca arundinacea Schreb.) in Australia. *Crop Pasture Sci* 2014;65:747.
6. Milne G. Management in New Zealand, Australia, and South America. In: Fribourg H, Hannaway D and West C (eds). *Tall Fescue for the Twenty-First Century*. Madison, WI: American Society of Agronomy. Crop Science Society of America: Soil Science Society of America; 2009. pp. 115–116.
7. Guo J, McCulley RL, McNear DH. Tall fescue cultivar and fungal endophyte combinations influence plant growth and root exudate composition. *Front Plant Sci* 2015;6:183.
8. Melchior EA, Smith JK, Schneider LG, Mulliniks JT, Bates GE, *et al*. Effects of red clover isoflavones on tall fescue seed fermentation and microbial populations in vitro. *PLoS One* 2018;13:e0201866.
9. Young CA, Charlton ND, Takach JE, Swoboda GA, Trammell MA, *et al*. Characterization of Epichloë coenophiala within the US: are all tall fescue endophytes created equal? *Front Chem* 2014;2:95.
10. Andrae JCL. Is MaxQ Tall Fescue Economical for Cow Calf Producers? University of Georgia Extension Publications. 2004. http://hdl.handle.net/10724/34327
11. Campbell BT, Kojima CJ, Cooper TA, Bastin BC, Wojakiewicz L, *et al*. A single nucleotide polymorphism in the dopamine receptor D2 gene may be informative for resistance to fescue toxicosis in angus-based cattle. *Anim Biotechnol* 2014;25:1–12.
12. Bastin BC, Houser A, Bagley CP, Ely KM, Payton RR, *et al*. A polymorphism in XKR4 is significantly associated with serum prolactin concentrations in beef cows grazing tall fescue. *Anim Genet* 2014;45:439–441.

13. Ayers AW, Hill NS, Rottinghaus GE, Stuedemann JA, Thompson FN, *et al*. Ruminal metabolism and transport of tall fescue ergot alkaloids. *Crop Sci* 2009;49:2309–2316.
14. Mote RS, Hill NS, Skarlupka JH, Turner ZB, Sanders ZP, *et al*. Response of beef cattle fecal microbiota to grazing on toxic tall fescue. *Appl Environ Microbiol* 2019;85:15.
15. Mote RS, Hill NS, Skarlupka JH, Tran VT, Walker DI, *et al*. Toxic tall fescue grazing increases susceptibility of the Angus steer fecal microbiota and plasma/urine metabolome to environmental effects. *Sci Rep* 2020;10:2497.
16. Chai J, Alrashedi S, Coffey K, Burke JM, Feye K, *et al*. Endophyte-infected tall fescue affects rumen microbiota in grazing ewes at gestation and lactation. *Front Vet Sci* 2020;7:544707.
17. de Oliveira Franco M, Detmann E, de Campos Valadares Filho S, Batista ED, de Almeida Rufino LM, *et al*. Intake, digestibility, and rumen and metabolic characteristics of cattle fed low-quality tropical forage and supplemented with nitrogen and different levels of starch. *Asian-Australas J Anim Sci* 2017;30:797–803.
18. Wright A-DG, Auckland CH, Lynn DH. Molecular diversity of methanogens in feedlot cattle from Ontario and Prince Edward Island, Canada. *Appl Environ Microbiol* 2007;73:4206–4210.
19. Liu J, Zhang M, Xue C, Zhu W, Mao S. Characterization and comparison of the temporal dynamics of ruminal bacterial microbiota colonizing rice straw and alfalfa hay within ruminants. *J Dairy Sci* 2016;99:9668–9681.
20. Nkrumah JD, Okine EK, Mathison GW, Schmid K, Li C, *et al*. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. *J Anim Sci* 2006;84:145–153.
21. USDA E. Cattle - Beef: Sector at a glance 2020; 2020. https://www.ers.usda.gov/topics/animal-products/cattle-beef/sector-at-a-glance/#:~:text=Cattle%20production%20is%20the%20most,for%20agricultural%20commodities%20in%202019
22. Bar Diamond I. Bovine surgery for fistulation of the rumen and cannula placement; 2011. https://bardiamond.com/animal-care-surgery-guides/
23. Bouton JH, Latch GCM, Hill NS, Hoveland CS, McCann MA, *et al*. Reinfection of tall fescue cultivars with non-ergot alkaloid–producing endophytes. *Agron J* 2002;94.
24. Sambrook J, Fritsch ETM. *Molecular Cloning: a Laboratory Manual*. 2nd ed. New York, NY: Cold Spring Harbor Laboratory Press; 1989.
25. Lane DJ. 16S/23S rRNA Sequencing. In: Stackebrandt E and Goodfellow M (eds). *Nucleic Acid Techniques in Bacterial Systematic*. New York: John Wiley and Sons; 1991. pp. 115–175.
26. Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, *et al*. Improved bacterial 16S rRNA Gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems* 2016;1:e00009-15.
27. Bolyen E, Rideout JR, Dillon MR, Abnet CC, Al-Ghalith GA. QIIME 2:reproducible, interactive, scalable, and extensible microbiome datascience. *PerrJ Preprints* 2018;6:e27295v2. DOI: 10.7287/peerj.preprints.27295v2 [Preprint].
28. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, *et al*. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;13:581–583.
29. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 2016;4:e2584.
30. Henderson G, Yilmaz P, Kumar S, Forster RJ, Kelly WJ, *et al*. Improved taxonomic assignment of rumen bacterial 16S rRNA sequences using a revised SILVA taxonomic framework. *PeerJ* 2019;7:e6496.
31. R-Core-Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: Foundation for Statistical Computing; 2012.
32. Ssekagiri AT, Sloan W, Zeeshan IU. *microbiomeSeq: an R package for analysis of microbial communities in an environmental context*. Entebbe, Uganda: ISCB Africa ASBCB Conference; 2017.
33. Lê Cao K-A, Costello M-E, Lakis VA, Bartolo F, Chua X-Y, *et al*. MixMC: A Multivariate Statistical Framework to Gain Insight into Microbial Communities. *PLoS One* 2016;11:e0160169.
34. Aitchison J. The statistical analysis of compositional data. *Journal of the Royal Statistical Society: Series B (Methodological)* 1982;44:139–160.
35. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;8:e61217.
36. Oksanen J, Blanchet F, Firiendly M, Kindt R, Laegendre P, *et al*. vegan: Community Ecology Package. R package version 2.5-6. 2019; 2019. https://CRAN.R-project.org/package=vegan
37. Lahti L, Sudarshan G, Obechain R, JS V, Blake T, *et al*. Tools for microbiome analysis in R. Microbiome package version.19.12; 2017. http://microbiome.github.com/microbiome
38. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:12.
39. Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* 1952;47:583–621.
40. Wilcoxon F. Individual Comparisons by Ranking Methods. *Biometrics Bulletin* 1945;1:80.
41. Harlow BE, Goodman JP, Lynn BC, Flythe MD, Ji H, *et al*. Ruminal tryptophan-utilizing bacteria degrade ergovaline from tall fescue seed extract. *J Anim Sci* 2017;95:980–988.
42. Koester LR, Poole DH, Serão NVL, Schmitz-Esser S. Beef cattle that respond differently to fescue toxicosis have distinct gastrointestinal tract microbiota. *PLoS One* 2020;15:e0229192.
43. Petri RM, Schwaiger T, Penner GB, Beauchemin KA, Forster RJ, *et al*. Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well as during and after an acidotic challenge. *PLoS One* 2013;8:e83424.
44. Li RW, Wu S, Baldwin RL 6th, Li W, Li C. Perturbation dynamics of the rumen microbiota in response to exogenous butyrate. *PLoS One* 2012;7:e29392.
45. Azad E, Derakhshani H, Forster RJ, Gruninger RJ, Acharya S, *et al*. Characterization of the rumen and fecal microbiome in bloated and non-bloated cattle grazing alfalfa pastures and subjected to bloat prevention strategies. *Sci Rep* 2019;9:4272.
46. Bowen JM, McCabe MS, Lister SJ, Cormican P, Dewhurst RJ. Evaluation of Microbial Communities Associated With the Liquid and Solid Phases of the Rumen of Cattle Offered a Diet of Perennial Ryegrass or White Clover. *Front Microbiol* 2018;9:2389.
47. Min BR, Gurung N, Shange R, Solaiman S. Potential role of rumen microbiota in altering average daily gain and feed efficiency in meat goats fed simple and mixed pastures using bacterial tag-encoded FLX amplicon pyrosequencing1. *J Anim Sci* 2019;97:3523–3534.
48. Noel SJ, Attwood GT, Rakonjac J, Moon CD, Waghorn GC, *et al*. Seasonal changes in the digesta-adherent rumen bacterial communities of dairy cattle grazing pasture. *PLoS One* 2017;12:e0173819.
49. Pitta DW, Pinchak WE, Dowd S, Dorton K, Yoon I, *et al*. Longitudinal shifts in bacterial diversity and fermentation pattern in the rumen of steers grazing wheat pasture. *Anaerobe* 2014;30:11–17.
50. Lourenco JM, Kieran TJ, Seidel DS, Glenn TC, Silveira MF da, *et al*. Comparison of the ruminal and fecal microbiotas in beef calves supplemented or not with concentrate. *PLoS One* 2020;15:e0231533.
51. Parmar NR, Nirmal Kumar JI, Joshi CG. Exploring diet-dependent shifts in methanogen and methanotroph diversity in the rumen of Mehsani buffalo by a metagenomics approach. *Frontiers in Life Science* 2015;8:371–378.
52. Koike S, Kobayashi Y. Fibrolytic Rumen Bacteria: Their Ecology and Functions. *Asian Australas J Anim Sci* 2009;22:131–138.
53. Miyazaki K, Hino T, Itabashi AH. Effects of extracellular pH on the intracellular pH and membrane potential of cellulolytic ruminal

bacteria, Ruminococcus albus, Ruminococcus flavefaciens, and Fibrobacter succinogenes. *J Gen Appl Microbiol* 1992;38:567–573.

54. Ogunade I, Pech-Cervantes A, Schweickart H. Metatranscriptomic analysis of sub-acute ruminal acidosis in beef cattle. *Animals (Basel)* 2019;9:E232.
55. Majak W, Cheng KJ. Identification of rumen bacteria that anaerobically degrade aliphatic nitrotoxins. *Can J Microbiol* 1981;27:646–650.
56. Martinez-Fernandez G, Denman SE, Cheung J, McSweeney CS. Phloroglucinol degradation in the rumen promotes the capture of excess hydrogen generated from methanogenesis inhibition. *Front Microbiol* 2017;8:1871.
57. Joblin KN, Naylor GE. The ruminal mycoplasmas: a review. *Journal of Applied Animal Research* 2002;21:161–179.
58. Ungerfeld EM. Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Front Microbiol* 2020;11:589.
59. Boone DR, Whitman WB, Rouviere P. Diversity and taxonomy of methanogens. In: Ferry JG (eds). *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics*. New York, NY: Chapman and Hall; 1993. pp. 35–80.
60. Mukhopadhyay B, Purwantini E, Conway de Macario E, Daniels L. Characterization of aMethanosarcina strain isolated from goat feces, and that grows on H2-CO2 only after adaptation. *Current Microbiology* 1991;23:165–173.
61. Xie X, Yang C, Guan LL, Wang J, Xue M, *et al*. Persistence of cellulolytic bacteria *Fibrobacter* and *Treponema* after short-term corn stover-based dietary intervention reveals the potential to improve rumen fibrolytic function. *Front Microbiol* 2018;9:1363.
62. Kudo H, Cheng KJ, Costerton JW. Interactions between *Treponema bryantii* and cellulolytic bacteria in the in vitro degradation of straw cellulose. *Can J Microbiol* 1987;33:244–248.
63. Hannah SM, Paterson JA, Williams JE, Kerley MS, Miner JL. Effects of increasing dietary levels of endophyte-infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. *J Anim Sci* 1990;68:1693–1701.
64. Bush LP, Burton H, Boling JA. Activity of tall fescue alkaloids and analogues in in vitro rumen fermentation. *J Agric Food Chem* 2002;24:869–872.
65. Silley P. The effect of plant alkaloids on *in vitro* rumen microbial fermentation . *J Appl Bacteriol* 1986;60:45–49.
66. Godoy-Vitorino F, Goldfarb KC, Karaoz U, Leal S, Garcia-Amado MA, *et al*. Comparative analyses of foregut and hindgut bacterial communities in hoatzins and cows. *ISME J* 2012;6:531–541.
67. Bergmann GT. Microbial community composition along the digestive tract in forage- and grain-fed bison. *BMC Vet Res* 2017;13:253.
68. Mao S, Zhang M, Liu J, Zhu W. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. *Sci Rep* 2015;5:16116.
69. De Lorme MJM, Lodge-Ivey SL, Craig AM. Physiological and digestive effects of Neotyphodium coenophialum-infected tall fescue fed to lambs. *J Anim Sci* 2007;85:1199–1206.