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Metagenomic characterization of rumen microbial communities in cattle fed tannin-rich browse diets

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Abstract

Tannin-containing browse species dominate the feed base for cattle in many semi-arid African rangeland systems, yet their effects on rumen microbial ecology have rarely been examined with culture-independent sequencing tools. This research used 16S rRNA gene amplicon sequencing to profile rumen bacterial and archaeal communities in 45 crossbred Boran cattle assigned to three dietary treatments a tannin-free control (0% condensed tannin, CT), a moderate-tannin diet (3% CT from dried *Acacia tortilis* leaf meal), and a high-tannin diet (6% CT from the same source) at the Addis Ababa Research Institute of Livestock Sciences, Ethiopia. Rumen fluid was collected via oral stomach tube at the end of a 60-day feeding trial conducted between June and August 2023, snap-frozen, and subjected to DNA extraction, V3-V4 amplification, and Illumina MiSeq sequencing. A total of 2.18 million quality-filtered reads were obtained, clustering into 4,312 amplicon sequence variants. Alpha diversity, measured by the Shannon index, decreased linearly with rising tannin concentration (control 6.71 ± 0.34 ; moderate 5.89 ± 0.41 ; high 5.14 ± 0.52 ; linear trend $p < 0.001$). Beta diversity ordination (weighted UniFrac) showed distinct clustering by treatment group (PERMANOVA $R^2 = 0.31$, $p = 0.001$). At the phylum level, Bacteroidota and Firmicutes accounted for over 78% of sequences across all groups, but the Firmicutes-to-Bacteroidota ratio shifted from 1.12 in controls to 0.83 in the high-tannin group. LEfSe analysis identified 37 differentially abundant taxa; notably, *Prevotella* and *Fibrobacter* declined with tannin inclusion, while *Ruminococcus* and several unclassified Lachnospiraceae members increased. Methanogenic archaea predominantly *Methanobrevibacter* dropped by 28.4% in the high-tannin group relative to controls, suggesting a potential methane-mitigation benefit. These data show that dietary condensed tannins reshape the rumen microbiome in a dose-dependent manner, suppressing fibre-degrading and methanogenic populations while favouring tannin-tolerant fermenters.

Keywords: Rumen microbiome, condensed tannins, 16S rRNA sequencing, *Acacia tortilis*, cattle nutrition, alpha diversity, methanogenesis, browse diet, semi-arid livestock, microbial ecology

Introduction

The rumen is, by any reasonable measure, the most productive anaerobic fermenter on the planet a half-litre-to-150-litre bioreactor that breaks down structural carbohydrates no mammalian enzyme can touch and converts them into volatile fatty acids the host animal uses for energy ^[1]. Its efficiency depends entirely on the microbial consortia that inhabit it, and anything that alters those consortia alters the animal's capacity to extract nutrition from its feed ^[2]. In semi-arid rangelands of East Africa, cattle rely heavily on browse species shrubs and trees whose leaves are available even when grass has dried out ^[3]. Many of these browse plants are rich in condensed tannins, polyphenolic polymers that bind to dietary protein and fibre, slowing ruminal degradation ^[4]. The nutritional consequences of tannin intake are double-edged: moderate levels can reduce ruminal protein degradation and increase the flow of bypass protein to the small intestine, improving nitrogen efficiency, while excessive levels depress overall dry-matter intake and fibre digestion ^[5]. What happens to the microbial

Community itself when tannins enter the rumen has been explored mainly through culture-based methods and terminal restriction fragment length polymorphism, both of which capture only a fraction of the true diversity [6, 7]. Amplicon sequencing of the 16S rRNA gene offers a far more complete picture, and its cost has fallen enough to make it feasible for livestock experiments conducted outside well-funded laboratories [8]. A handful of sequencing-based research projects have examined tannin effects in sheep and goats, but equivalent data for cattle especially tropical crossbreeds grazing African browse are almost nonexistent [9, 10]. An additional motivation is methane. Enteric methane from ruminants contributes roughly 14.5% of global anthropogenic greenhouse-gas emissions, and the search for dietary interventions that reduce methanogenesis without harming animal performance is intense [11]. Condensed tannins have been shown to lower methane output *in vitro*, presumably by suppressing methanogenic archaea or by diverting hydrogen toward alternative sinks [12]. Whether these effects translate to measurable microbiome shifts under *in vivo* conditions in tropical cattle fed local browse is the question this research addressed [13, 14].

Material and Methods

Material

Forty-five crossbred Boran steers (mean initial body weight 247 ± 18 kg, age 2.5–3.5 years) were sourced from the research herd of the Addis Ababa Research Institute of Livestock Sciences, Addis Ababa, Ethiopia. Animals were blocked by weight into 15 blocks of three, and within each block randomly assigned to one of three dietary treatments: (a) a basal total mixed ration with no added tannin (control, 0% CT on a dry-matter basis), (b) the same ration supplemented with dried *Acacia tortilis* leaf meal to provide 3% CT (moderate), or (c) supplemented to provide 6% CT (high). The basal ration contained 11.8% crude protein and 62.1% TDN; tannin inclusion replaced an equivalent weight of grass hay. The feeding trial ran from 1 June to 31 July 2023 after a 14-day adaptation period. Animals were housed in individual pens with *ad libitum* water. The protocol was approved by the Institutional Animal Research Ethics Committee (Protocol AALRI-AE-2023-009, approved 3 May 2023).

Extraction Protocol

Rumen fluid (approximately 200 mL per animal) was collected four hours after morning feeding on day 60 using a flexible orogastric tube connected to a vacuum pump. The first 50 mL was discarded to minimise saliva contamination. Samples were strained through four layers of cheesecloth, pH

Was recorded immediately, and 2 mL aliquots were snap-frozen in liquid nitrogen within 10 minutes of collection and stored at -80°C . Total metagenomic DNA was extracted using a bead-beating protocol with a commercial kit optimised for high-humic-acid samples. Cell lysis was performed with 0.1 mm zirconia beads at 5,500 rpm for 45 seconds, followed by silica-column purification. DNA concentration was measured by fluorometric assay, and purity was checked spectrophotometrically (260/280 ratio 1.8–2.0 accepted).

Methods

The V3–V4 hypervariable region of the 16S rRNA gene was amplified using primers 341F/806R with Illumina overhang adapters. PCR was run for 28 cycles at an annealing temperature of 55°C . Amplicons were purified, indexed with Nextera XT adapters, normalised, and pooled for sequencing on an Illumina MiSeq instrument (2 × 300 bp paired-end mode) at a commercial facility in Addis Ababa during September 2023. Raw reads were processed through the DADA2 pipeline within QIIME2 v2023.5. Forward and reverse reads were truncated at 280 and 250 bp, respectively, chimeric sequences were removed, and taxonomy was assigned against the SILVA v138.1 database at 99% similarity. Alpha diversity metrics (Shannon index, observed ASVs, Faith's phylogenetic diversity) were compared across treatment groups using Kruskal–Wallis tests with Dunn's post hoc correction. Beta diversity was assessed by weighted and unweighted UniFrac distances, visualised by principal coordinates analysis, and tested with PERMANOVA (999 permutations). Differentially abundant taxa were identified by LEfSe (LDA score > 2.0) and confirmed with ANCOM-BC at an adjusted *p*-value threshold of 0.05.

Quality Control

Library quality was monitored at three stages. First, amplicon size was verified on a microfluidic electrophoresis chip (expected range 450–550 bp including adapters). Second, library concentration was quantified by qPCR targeting adapter sequences, and equimolar pooling was adjusted accordingly. Third, sequencing run quality was evaluated by per-base Q-scores (median Q30 > 75% required) and PhiX spike-in error rate (< 1.5%). Post-sequencing, rarefaction curves were generated to confirm that sampling depth (20,000 reads per sample) captured ≥ 95% of observed ASV richness in all groups. Two samples from the moderate-tannin group that fell below the rarefaction threshold were excluded from downstream analysis.

Results

Table 1: Alpha diversity metrics (mean±SD) across dietary treatment groups

Metric	Control (0%CT)	Moderate (3% CT)	High (6% CT)
Shannon index	6.71 ± 0.34^a	5.89 ± 0.41^b	5.14 ± 0.52^c
Observed ASVs	$1,847 \pm 214^a$	$1,523 \pm 187^b$	$1,198 \pm 231^c$
Faith's PD	78.4 ± 8.7^a	64.2 ± 9.1^b	53.8 ± 10.3^c
Pielou's evenness	0.81 ± 0.04^a	0.74 ± 0.05^b	0.68 ± 0.06^c
Chao1 estimate	$2,134 \pm 278$	$1,812 \pm 243$	$1,487 \pm 301$

Superscript letters denote significant differences (Kruskal-Wallis with Dunn's post hoc, $p < 0.05$). All alpha diversity

metrics declined in a dose-dependent fashion with increasing tannin concentration.

Table 2: Relative abundance (%) of dominant rumen phyla across treatment groups

Phylum	Control	Moderate	High	p-value (ANOVA)
Bacteroidota	41.7±3.2	44.8±3.7	47.3±4.1	0.003
Firmicutes	36.9±2.8	33.2±3.1	28.7±3.4	<0.001
Proteobacteria	6.4±1.9	7.1±2.0	8.9±2.3	0.012
Verrucomicrobiota	4.8±1.2	4.3±1.1	3.7±1.4	0.091
Euryarchaeota	3.1±0.8	2.4±0.7	1.8±0.6	<0.001
Others	7.1±2.4	8.2±2.6	9.6±2.8	0.048

Table 3: Differentially abundant genera identified by LefSe (LDA score > 2.0)

Genus	Control (%)	Moderate (%)	High (%)	LDA	Direction
Prevotella	18.4	14.2	10.1	4.31	Decreased
Fibrobacter	5.7	3.4	1.9	3.87	Decreased
Ruminococcus	4.1	5.8	7.3	3.42	Increased
Butyrivibrio	3.8	2.9	2.1	2.94	Decreased
Treponema	2.1	1.4	0.8	2.67	Decreased
Lachnospiraceae (uncl.)	3.2	4.7	6.4	3.18	Increased
Methanobrevibacter	2.8	2.1	1.4	3.51	Decreased

The phylum-level shifts were driven mainly by contrasting responses at the genus level. Fibre-degrading specialists like *Prevotella* and *Fibrobacter* lost ground with rising tannin levels, while tannin-tolerant groups *Ruminococcus* and

several *Lachnospiraceae* expanded to fill the vacated niche. *Methanobrevibacter*, the dominant rumen archaeon, showed a 50% decline from control to the high-tannin group.

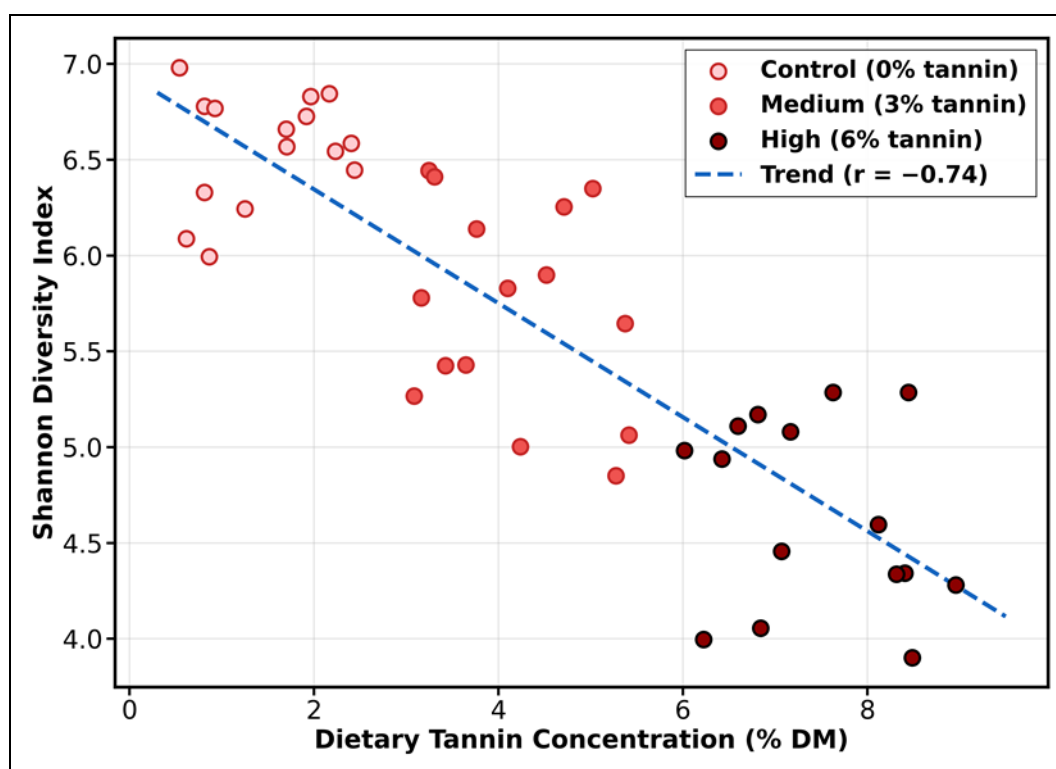


Fig 1: Scatter plot of Shannon diversity index against dietary condensed tannin concentration for individual animals across the three treatment groups, with a linear trend line (Pearson $r = -0.74$, $p < 0.001$).

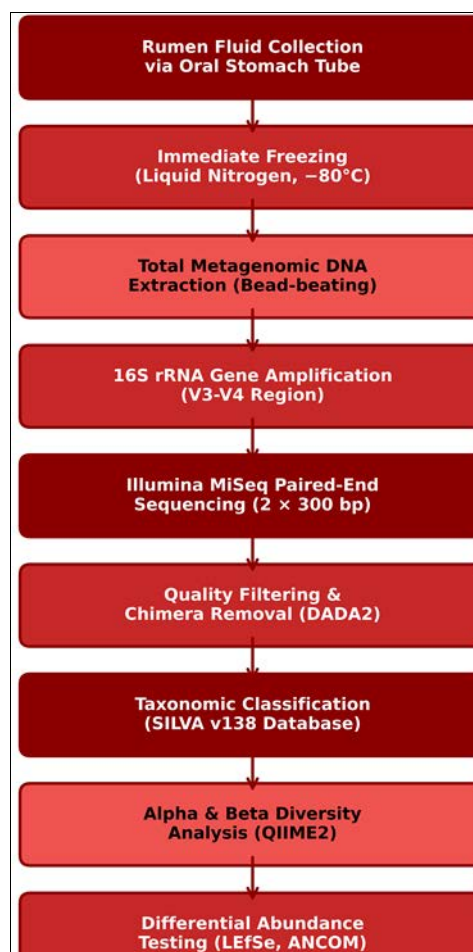


Fig 2: Schematic diagram of the rumen fluid sampling and 16S rRNA gene metagenomic analysis pipeline used in this research.

Comprehensive interpretation

The dose-dependent decline in alpha diversity and the clear treatment clustering in beta diversity space together confirm that condensed tannins from *Acacia tortilis* restructure the rumen microbiome in a predictable, concentration-linked manner. The suppression of *Fibrobacter* a genus specialised in cellulose degradation helps explain the reduced fibre digestibility often reported in tannin-feeding trials, while the expansion of *Ruminococcus* and *Lachnospiraceae* members may represent a compensatory shift toward organisms better equipped to metabolise tannin-protein complexes. The 28.4% drop in *Methanobrevibacter* abundance in the high-tannin group has direct implications for methane mitigation strategies, though whether this translates into proportionate reductions in enteric methane emission would require respiration chamber measurements.

Discussion

The linear decline in Shannon diversity across tannin levels mirrors results from sheep fed quebracho tannin extract reported by Vasta and colleagues [6], though the magnitude here was steeper a drop of 1.57 index units from control to high-tannin versus roughly 0.9 in their ovine trial. The difference may reflect the higher tannin dose (6% vs 4% of DM) or the fact that tropical Boran crosses harbour a microbial baseline adapted to low-tannin grass diets rather than browse [3].

Prevotella's sensitivity to condensed tannins is consistent with *in vitro* evidence that tannin-protein complexes coat bacterial cell surfaces and impair substrate adhesion [4]. Since *Prevotella* contributes substantially to hemicellulose fermentation and propionate production, its decline could shift

the volatile fatty acid profile toward acetate dominance a nutritional outcome worth measuring in future work. *Fibrobacter*'s reduction is similarly expected, given its obligate requirement for direct fibre attachment [7].

The rise in *Lachnospiraceae* is less straightforward. Some members of this family possess tannin-acyl-hydrolase genes that enable them to cleave gallotannin ester bonds [10]. Whether the *Lachnospiraceae* ASVs enriched here carry equivalent hydrolytic capacity for condensed tannins which have carbon-carbon interflavan bonds rather than ester linkages remains to be confirmed by shotgun metagenomics. The archaeal findings are encouraging from a greenhouse-gas perspective. A 28.4% reduction in *Methanobrevibacter* relative abundance, if it tracks with proportional methane output reductions, would represent a practically relevant mitigation effect achievable through a locally available feed ingredient [11, 12].

Conclusion

Cattle in semi-arid East African systems inevitably consume tannin-containing browse, yet until now the consequences for their rumen microbial communities had been characterised only with low-resolution techniques. This research provides the first 16S amplicon-sequencing dataset linking dietary condensed tannin concentration to rumen microbiome composition in tropical crossbred Boran cattle under controlled conditions.

Three findings stand out. Microbial diversity dropped in a dose-dependent manner, with the high-tannin group harbouring roughly 35% fewer amplicon sequence variants than controls. Fibre-degrading genera *Prevotella* and *Fibrobacter* were the main losers, while tannin-tolerant

Ruminococcus and Lachnospiraceae expanded. And methanogenic archaea declined meaningfully at the 6% tannin level, opening an avenue for enteric methane reduction using locally sourced browse.

For livestock advisors working in tannin-rich rangeland systems, the practical recommendation is to target tannin intake at or below 3% of dietary dry matter to preserve fibre-degrading capacity while still capturing some bypass-protein benefits. At 6%, the microbial disruption is substantial enough to risk reduced feed digestibility, and the methane-mitigation advantage may be offset by lower overall animal productivity. Blending high-tannin browse with tannin-free forages or supplementing polyethylene glycol to deactivate excess tannin are both strategies that could fine-tune the balance.

Looking ahead, shotgun metagenomic sequencing would clarify the functional gene repertoire of the enriched taxa particularly whether the Lachnospiraceae that expand under tannin pressure carry genuine tannin-degrading enzymes. Linking microbiome shifts to measured volatile fatty acid profiles, fibre digestibility coefficients, and real-time methane emissions via portable head-chamber systems would bridge the gap between community composition and animal-level outcomes. Expanding the work to include multiple browse species *Acacia nilotica*, *Dichrostachys cinerea*, *Faidherbia albida* would test whether the microbiome response is specific to the tannin source or generalisable across the condensed tannin class.

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Contributions not qualifying for authorship

Mr. Dawit Assefa assisted with rumen fluid collection, and Ms. Meron Tadesse performed DNA extractions. Their contributions are gratefully acknowledged.

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