

LIGHTNING TALK:

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Shining a light on prebiotic-probiotic fructan-microbial interactions in the rumen

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Inulin, and fructooligosaccharides (FOS) derived from inulin, are fructan-based prebiotics for humans, livestock, and companion animals. Inulin elicits a bifidogenic effect by selectively promoting the growth and abundance of *Bifidobacterium* species within an intestinal microbiota. Structurally, inulin contains β -2,1 glycosidic linkages between its fructose monomers; in contrast levan is another commercially available fructan with β -2,6 linkages. Apart from these well studied fructans, more complex types such as graminans exist, but their role as a prebiotic has not been extensively studied. Graminan is present during the immature growth phase of cereal crops where they serve as a temporary storage carbohydrate before starch accumulation begins, and contains both β -2,1 and β -2,6 glycosidic linkages. While inulin is widely advertised as a prebiotic for companion animals, graminans may serve as new, low-cost, and convenient prebiotic for livestock, such as cattle.

To compare the prebiotic potential of graminans to conventional fructans, rumen-isolated *Bifidobacterium* and *Lactobacillus* strains were first assessed for their potential to metabolize levan, inulin, or inulin-derived FOS, as a sole carbon source. Out of the six strains tested, *L. vitulinus* (ATCC 27783) was the only strain that was able to metabolize all three types of commercial fructans, whereas *B. boum* (ATCC 27917) and *B. merycicum* (ATCC 49391) could only utilize inulin and FOS. Fluorescent polysaccharide (FLA-PS) versions of levan and inulin

were created to 'directly' visualize and quantify fructan-microbial interactions in pure cultures and rumen samples. To explore the potential prebiotic effect of inulin within a rumen microbial community, rumen samples \pm 1.5% or 3% inulin were analysed *in vitro* for changes to microbial composition and fermentation 48 hours after prebiotic enrichment. 16S metagenomics analysis revealed that the abundance of *Bifidobacterium* and *Lactobacillus* OTUs increased within inulin-enriched rumen samples relative to the control. Additionally, there was an overall increase in short chain fatty acid production, suggesting that inulin supplementation alters ruminal fermentation.

Next, to study the metabolism of graminans, immature barley and wheat crops were collected and assessed for the quantity of fructans in the kernel, stem, and leaf fractions. The majority of fructan within each crop resides in the stem and kernel fractions. From these fractions, fructans were isolated and analyzed by HPAEC-PAD to determine peak structures and degrees of polymerization. The ability of *L. vitulinus*, *B. boum*, and *B. merycicum* to utilize these purified fructans was then assessed. Each strain displayed a more efficient utilization graminans compared to inulin-derived FOS. Based on these initial results, it is suggested that graminans will likely have a similar bifidogenic effect on the rumen microbiome, holding promising potential for being a prebiotic candidate. Looking forward, combining the use of next-generation microbial ecology methods, such as FLA-PS, coupled to more common methods to analyze microbial communities, such as FISH and 16S rRNA amplicon sequencing, will help design suitable prebiotic candidates to improve cattle performance.