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## Lysolecithin addition to ruminant feed: mode of action insights

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Lysolecithins (LL) are world-widely recognized for aiding nutrient digestion and absorption of monogastric animals, with recent evidence suggesting benefits in ruminant diet. A lot of theories have been put forward to explain these benefits in ruminants, but, up till now, there is no scientific "mode-of-action" evidence available. In order to bridge this gap, a LLbased product (soy bean LL with 11% lysophospholipids; Kemin Animal Nutrition and Health, Belgium) was incubated for various durations in an in vitro rumen batch system. The rumen fluid/buffer ratio was set at 1:4, and the inoculum was obtained from 3 rumen-fistulated dairy cows on a basal lactation diet. Analyses included gas chromatography, p-NMR, and 16S metagenomics sequencing, assessing volatile fatty acid (VFA) production, phospholipid characterization, and rumen microbiome. There was a control treatment and a LL treatment. For the latter treatment, 2.5 g of test product (soy bean LL) was added per 500 ml. Each treatment had 6 replicates. Statistical procedures were carried out in JMP®, Version 15.0.0 SAS Institute Inc., Cary, NC, 1989-2019. The effect of treatments on variable parameters was analyzed with ANOVA. Post-hoc comparisons between treatments were investigated by Duncan's test. Results showed that 73.4% of the total LL remained intact after 4h, 43.6% after 8h, and 8.4% after 24h of rumen incubation. Total VFA production tended to be higher in treated flasks compared to control at all incubation times (29.1%, 24.7%, 12.5% higher at 4h, 8h, and 24h, respectively with P<0.1). The effect seen on increased propionate, caproate and butyrate production was significant (P<0.05). Absolute methane production was unaffected, while ratio of methane:VFA decreased with treatment (P=0.01). Abundances of Ruminococcaceae, Desulfovibrionales, and Cyanobacteria were significantly increased by treatment (P<0.05), suggesting enhanced fiber, sulfur, and nitrogen utilization. Microbial diversity (based on number of ASV) was also higher in the treatment compared to the control group. Overall, the data reveal that soy-bean based LL in the in vitro fermentation model itself could increase VFA production, decrease methane: VFA production ratio and alter the in vitro rumen microbiome diversity. These effects could potentially lead to better animal performance and better production of milk components.

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