

Japan). Three hundred and seventy-five lactating cows (132 primiparous, 243 multiparous; BW = 621 ± 76 kg; DIM = 145 ± 93 , yield = 42.1 ± 8.1 kg/d) were randomly allocated to 3 treatments and fed a common TMR containing 15.4% CP, 39.7% NDF and 1.57 Mcal of NEI/kg. Treatments consisted of no supplementation (T0), 1.5×10^5 CFU/g (T1), and 3×10^5 CFU/g (T2) supplementation with *B. subtilis* C-3102. Calsporin® was supplemented individually during milking using a precision feeding system. At each milking, cows received either 100 g of barley (T0), or 100–150 g of barley containing Calsporin® to supplement either 0.3 (T1) or 0.6 g/cow/day (T2) for 105 d. Cows are milked 3 times a day. Milk production was recorded daily. Thirty cows (10 from each treatment) were sampled for rumen contents at 42 and 84 d using an esophageal tube after the morning milking. Rumen pH and VFA concentrations determined. Data were analyzed using a mixed-effects model with week as a repeated measure. Cow was the experimental unit ($n = 125$). Primiparous cows on T2 produced more ($P < 0.01$) milk after wk 6 of study (35.6 ± 0.82 kg/d) than primiparous cows on T1 and T0 (34.2 ± 0.82 kg/d), whereas multiparous cows on both T1 and T2 (38.5 ± 0.82 kg/d) produced ($P < 0.05$) more milk than multiparous cows on T0 (36.1 kg/d) after 3 wk of study. Changes in rumen fermentation profile were minor. Rumen molar proportions of propionate decreased ($P < 0.05$) at 84 d compared with d 42 in T1 and T2 (from 26.8 to $25.2 \pm 0.48\%$) compared with T0, which remained constant ($24.8 \pm 0.48\%$). Molar proportions of rumen butyrate decreased ($P < 0.05$) between 42 and 84 d in T0 (from 11.5 to $9.9 \pm 0.43\%$) compared with in T1 and T2, which remained constant ($10.5 \pm 0.43\%$). The supplementation of Calsporin® seems to exert a positive effect on milking persistency of dairy cows, with a positive response already obtained 1.5×10^5 CFU of *B. subtilis*/g. About 3 and 6 wk of exposure to treatments are needed for a milk response to become evident in multiparous and primiparous cows, respectively.

Key Words: probiotic, rumen, yield
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1642 Effect of *Enterococcus faecalis* SROD5 supplementation on microbial communities and quantities of in vitro rumen fermentation.

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Enterococcus faecalis is one of the beneficial microorganisms, which produces fumarate reductase that converts fumarate to succinate and reduces methane production in vitro. Hence, this study was conducted to determine the effect of *E. faecalis* SROD5 supplementation on archaeal diversity and microbial population. Fresh culture of *E. faecalis* SROD5 (7.5×10^8 cfu/ml) at different inclusion rates (0, 0.1%, 0.5%, and 1.0%) were investigated using in vitro rumen fermentation. Ruminant

samples were collected from cannulated Holstein Friesian cow and 40:60 rice straw to concentrate ratio were used as substrate at 1 g dry matter (DM) per 100 ml buffered rumen fluid. Samples from in vitro fermentation of 12 h incubation were used for determination of microbial community and quantity. Pyrosequencing of archaeal 16S rRNA gene showed that the number of operational taxonomic units (OTU) was highest in supplementation of 0.1% *E. faecalis* SROD5 (39). Shannon-Weaver index were comparable among control and treatments while Chao 1 was higher in 0.1% and 0.5% supplementation of *E. faecalis* SROD5 with 52 and 54, respectively. Meanwhile, alignment of archaeal reads showed that almost all retrieved from in vitro rumen fermenta samples fell into the phylum *Euryarchaeota*, which predominantly affiliated with family *Methanobacteriaceae* (97% to 99%) followed by *Methanomicrobiaceae*, and *Methanosarcinaceae*. Abundance of *Methanobrevibacter* was higher in non-supplementation of *E. faecalis* SROD5 with 96.54%. Higher abundance of *Methanomicrobium* was observed in 0.1% *E. faecalis* SROD5 supplementation while higher abundance of *Methanosphaera* and unclassified *Methanobacteriaceae* as well as the presence of *Methanimicrococcus* were observed in 0.5% *E. faecalis* SROD5. Supplementation of 0.1% *E. faecalis* had the highest quantities of total bacteria (2.59×10^8 copies/ml), total fungi (1.03×10^4 copies/ml), *Fibrobacter succinogenes* (1.62×10^5 copies/ml), and *Ruminococcus flavefaciens* (1.51×10^3 copies/ml) while the highest methanogen quantity was observed in non-supplementation of *E. faecium* with 2.74×10^1 copies/ml. Addition of *E. faecalis* SROD5 changed the archaeal communities of in vitro rumen fermenta. Supplementation of 0.1% *E. faecalis* SROD5 increased microbial population and decreased methanogen quantity.

Key Words: *Enterococcus faecalis* SROD5, in vitro, pyrosequencing
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1643 Effects of dietary neutral detergent fiber and starch ratio on rumen epithelial cell morphological structure and gene expression in dairy cows.

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Dietary neutral detergent fiber (NDF):starch ratio has been considered a potential indicator to reflect carbohydrate composition in diet formulation and could affect the composition and content of VFA in rumen of dairy cow. Rumen epithelial

papilla as small bumps of the rumen mucosal epithelium could broaden the surface of the rumen, which was beneficial for improving the absorption ability of nutrients, especially for VFA. This study was designed to investigate the effect of dietary NDF:starch ratio on rumen epithelial cell morphological structure and gene expression. Eight primiparous dairy cows including 4 rumen cannulated animals were assigned to 4 total mixed rations with NDF:starch ratios of 0.86, 1.18, 1.63, and 2.34 from T1 to T4 in a replicated 4×4 Latin square design. The duration of each period was 21 d including a 14 d adaptation period and a 7 d sampling period. Rumen epithelial papilla was collected from rumen cannulated cows. Morphological structure of rumen epithelial papilla was detected and several genes related to the absorption and metabolism of VFA and growth of rumen epithelial papilla cell were analyzed with quantitative real-time PCR, including NHE1, NHE3, NHE4, MCT1, MCT2, MCT4, Na/KAT-Pase, HMGCS, ACSS1, ACSS2, ACSS3, HMGCL, ACAT1, IGFBP3, IGFBP5, and IGFBP6. The results showed that the thickness of stratum spinosum and basale was linearly increased with increasing of dietary NDF:starch ratio (39.58^a , 42.84^a , 43.24^a , and 54.22^b mm for T1 to T4, $P = 0.02$), which indicated that surface of the rumen wall could be broadened and the absorption capability of VFA could be improved with the increasing dietary NDF:starch ratio. Expression of HMGCS as the limited enzyme in synthesis of ketone body metabolized by VFA was linearly downregulated ($P = 0.02$), while the expression of MCT2 positively correlated with the absorption capability of VFA was linearly upregulated with the dietary NDF:starch ratio increasing ($P < 0.01$). As dietary NDF:starch ratio increased, expression of IGFBP5 related to the growth of rumen epithelial papilla was downregulated ($P < 0.01$), while IGFBP6 expression was upregulated ($P < 0.01$), which were regulated by the short-chain fatty acids (SCFA) part of VFA. Dietary NDF:starch ratio significantly improved the thickness of stratum spinosum and basale of rumen epithelial papilla and regulated genes expression of VFA absorption and metabolism and growth of rumen epithelial cell, which may be indicative of an activated response of VFA absorption improvement with dietary NDF:starch ratio increasing.

Key Words: dairy cow, NDF:starch ratio, rumen epithelial cell morphological, gene expression
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- 1644 Rumen disappearance of capsaicin and dihydrocapsaicin in lactating dairy cows.** J. Oh^{*1}, D. M. Bravo², E. H. Wall², and A. N. Hristov¹, ¹The Pennsylvania State University, University Park, ²Pancosma, Geneva, Switzerland.

The objective of this study was to assess rumen disappearance rate and escape of the two main active compounds of *Capsicum* oleoresin, capsaicin (CAP), and dihydrocapsaicin (DHC), in lactating dairy cows. The study involved 4 early- to

late-lactation Holstein cows (24 ± 2.9 kg/d DMI; 38 ± 5.2 kg/d milk yield; 624 ± 28 kg BW) and consisted of 3, 10-d experimental periods. *Capsicum* oleoresin (CO) was administered into the rumen of the cows at 3 pulse-doses: 250, 500, and 1000 mg/cow. All cows received 250 mg CO in period 1500 mg in period 2, and 1000 mg in period 3. Chromium-EDTA was used as a rumen fluid phase passage rate marker. On day one of each experimental period, CO and Cr-EDTA solutions were administered intraruminally in each cow through the rumen cannula at the time of feeding (cows were fed once daily around 8 a.m.). Rumen fluid samples were collected at 0 (background), 0.5, 1, 2, 6, 12, and 24 h post-CO administration and analyzed for Cr, CAP, and DHC. Concentration data were fitted to a single exponential decay model using the NLIN procedure of SAS. The rate of degradation of CAP or DHC was found as: $k_{Cr} \div k_{CAP}$ or $k_{DHC} \times 100$, where k_{Cr} is the slope of decline in Cr concentration in ruminal fluid (on average 0.25 ± 0.06 h⁻¹) and k_{CAP} or k_{DHC} are slopes of decline in CAP and DHC concentrations. Rumen disappearance rates of CAP were 1.34 ± 0.711 , 0.91 ± 0.248 , and 1.50 ± 0.309 h⁻¹ for 250, 500, and 1000 mg CO, respectively. Rumen disappearance rates of DHC were 2.22 ± 1.018 , 1.82 ± 0.336 , and 1.62 ± 0.372 h⁻¹, respectively. Rumen escape of CAP was estimated at 15.4, 32.6, and 17.6%, respectively, and that of DHC was 31.7, 19.3, and 16.1%, respectively. Average concentration of DHC in fecal samples was 35.6 ± 6.05 ng/g with the 1000 mg dose, whereas capsaicin was not detected in feces. CAP and DHC concentrations were 361 ± 52.6 and 203 ± 27.9 ng/mL in milk, respectively, with the 1000 mg dose, and the compounds were not detected in milk from non-treated cows. In this study, rumen escape of CAP and DHC in lactating dairy cows was between 15.4 and 32.6%, depending on dose.

Key Words: capsaicin, dihydrocapsaicin, rumen disappearance
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- 1645 WS Effects of capsaicin source on blood capsaicin, glucose and insulin concentrations, rumen fermentation and nitrogen balance of sheep.** J. B. Alford¹, J. G. Castro^{*1}, E. R. Oosthuisen¹, S. L. Rosasco², R. D. Richins¹, E. J. Scholljegerdes¹, D. M. Hallford², and C. A. Loest¹, ¹New Mexico State University, Las Cruces, ²Animal and Range Science Dep., New Mexico State University, Las Cruces.

This study evaluated the bioavailability of ruminally-protected capsaicin, and potential effects on rumen microbial fermentation, diet digestibility, and N retention of sheep. Twenty-one wether lambs (36.1 ± 1.0 kg BW) were used in 2 experimental periods (19 d each) based on BW (9 and 12 lambs in period 1 and 2, respectively). From d 1 to 7 of each period, lambs were adapted to indoor individual pens, and then moved to metabolism crates from d 8 to 19. Lambs were fed twice daily alfalfa hay and 1 of 3 supplements containing