

T264 The use of flow cytometry to assess rumen bacteria in dairy heifers limit fed different forage to concentrate ratios with *Saccharomyces cerevisiae*. G. J. Lascano* and A. J. Heinrichs, *The Pennsylvania State University, University Park.*

Counting total viable bacteria using colony-unit forming assays lacks accuracy, as this method only includes culturable bacteria capable of initiating cell division. Thus, viable bacterial counts are often underestimated and total counts unknown. Both live and total bacteria populations can be enumerated using fluorescent characteristics of cell membranes and flow cytometry. The objective of this experiment was to investigate viable and total rumen bacteria counts using LIVE/DEAD Backlight kit (Invitrogen, Corp.) when 3 levels of forage:concentrate (F:C) were fed to heifers at restricted levels of intake with or without the addition of *Saccharomyces cerevisiae* (YC, Yea-Sacc¹⁰²⁶, Alltech, Inc). Three cannulated, post-pubertal Holstein heifers (age 18 ± 1 mo) were fed corn silage (CS)-based diets in a 3-period (35 d) Latin Square design. Heifers were fed F:C treatment diets for 21 d, followed by 14 d with YC added (1 g/kg, as fed). High concentrate (HC) TMR (40% CS, 60% grain; 12.6% CP, 25% NDF), medium concentrate (MC) TMR (60% CS, 40% grain; 12.3% CP, 28% NDF), and low concentrate (LC) TMR (80% CS, 20% grain; 12.4% CP, 35% NDF) were fed once/d. Rumen fluid was sampled at -2, 0, 2, 4, 6, 8, 10, 12 h after feeding. Samples were immediately stained with LIVE/DEAD kit and analyzed with a Coulter XL-MCL single laser flow cytometer (Beckman Coulter, Inc.). Mean live bacteria count was not different among treatments ($6.75, 4.77, 4.97 \times 10^{11} \pm 0.53 \times 10^{11}$ cells/mL; $P = 0.10$) for HC, MC and LC, but YC addition increased number of viable bacteria in all treatments ($P < 0.01$). There was also a ration by YC interaction with greatest differences in the HC diet ($P = 0.01$). Total live bacteria counts decreased after feeding ($P < 0.01$) and began to increase 4 h after feeding. We conclude that feeding different ratios of F:C did not change total viable bacteria population, but YC increased this count in all 3 diets in this experiment.

Key Words: Viable Bacteria Count, Flow Cytometry, Yeast Culture