Microbial rDNA sequences have been proposed as potential internal markers to determine microbial synthesis in the rumen. The objective of this experiment was to compare values of microbial growth determined using $^{15}$N as external marker with concentrations of microbial DNA in fermenters, and to assess if both procedures detected similar differences between diets and solid (SOL) and liquid (LIQ) digesta phases. Four Rusitec vessels were used in a crossover trial with 2 14-d periods. In each period, 2 fermenters received a 50:50 alfalfa hay:concentrate diet (MC) and 2 were fed a 15:85 barley straw:concentrate diet (HC). A solution of $^{15}$NH$_4$Cl was infused for 5 d before taken samples of SOL and LIQ digesta and isolation of bacterial pellets from both digesta phases to estimate microbial protein synthesis. Samples of SOL and LIQ digesta were simultaneously taken for DNA extraction and analysis of concentrations of total bacterial and protozoal DNA by quantitative PCR. Total microbial N (TMN) was calculated from the $^{15}$N enrichment in digesta and isolated bacterial pellets, and total microbial DNA (TMDNA) was calculated as the sum of bacterial DNA and protozoal DNA in both digesta fractions. There were no diet × digesta phase interactions ($P > 0.05$) with any marker. Both TMN and TMDNA were greater ($P < 0.001$) in MC-fermenters than in HC-fermenters (1.5 and 2.0 times greater for TMN and TMDNA, respectively). Values of TMN were greater ($P = 0.004$) in SOL than in LIQ digesta (108 and 89.7 mg N, respectively), whereas the opposite was found for TMDNA (3.37 and 13.1 mg DNA, respectively). There was no difference between diets ($P > 0.05$) in the contribution of SOL digesta to TMN (53.8 and 55.7% for MC and HC diets, respectively), but contribution of SOL digesta to TMDNA was greater in MC than in HC diet ($P = 0.039$; 24.5 and 11.5%, respectively). There was no relationship ($P > 0.05$) between TMN and TMDNA values, but a significant relationship was observed when only values in the LIQ digesta were considered ($P = 0.024$; $r = 0.821$). In summary, both markers detected similar differences between diets, but not between digesta phases.

Key Words: microbial growth, $^{15}$N, qPCR

T487 Microbial rDNA sequences as markers to determine microbial synthesis in Rusitec fermenters: A comparison with $^{15}$N. Cristina Saro$^2$, Maria Jose Ranilla$^{2,3}$, Ivan Mateos$^2$, Alexey Díaz$^2$, Jairo García$^2$, Maria Gracia de García$^2$, and Maria Dolores Carro$^1$, $^1$Technical University of Madrid, Madrid, Spain, $^2$University of León, León, Spain, $^3$IGM (CSIC-ULE), Grulleros, León, Spain.

Effect of chitosan in dairy cows diets on ruminal fermentation and milk yield and composition. Carlos Eduardo Cardoso Consentini$^1$, Elmesome Ferreira de Jesus$^2$, Pablo Gomes de Paiva$^2$, Tiago Antonio Del Valle$^1$, Gustavo Ferreira de Almeida$^1$, Artur Gabriel Brao Vilas Boas Costa$^1$, Fernanda Carolina Ramos dos Santos$^1$, Victor Chiaromi Galvão$^1$, and Francisco Palma Remió$^1$, $^1$School of Veterinary Medicine and Animal Science of USP, Pirassununga, São Paulo, Brazil, $^2$School of Agricultural and Veterinary Sciences of UNESP, Jaboticabal, São Paulo, Brazil.

In this study, we evaluate the effects of chitosan level for cows in late lactation on ruminal fermentation and milk yield and composition. Eight Holstein cows cannulated in the rumen (215.4 ± 60.9 DIM, 22.07 ± 5.32 kg of milk yield and 641.6 ± 41.06 kg of BW) in replicated 4 × 4 Latin squares, were fed the following diets: (CTR) control diet without addition of chitosan, CH175 mg/kg, CH150 mg/kg and 225 mg/kg of chitosan addition per kg BW. Each period had 14 d adaptation and 7 for collection data. Ruminal fluid was collected on 20th day of each period at 7 times to evaluate the effect of the diets on ruminal fermentation. Sampling of milk was done on 16 to 18th day of each period to evaluate the composition. The results of milk composition was subjected to ANOVA, while ruminal fermentation data were analyzed as repeated measures by PROC MIXED of SAS. Chitosan changed ruminal fermentation profile, increasing rumen propionate ($P < 0.05$) without shift acetate and total VFA concentrations ($P > 0.05$). However, chitosan decreased linearly acetate:propionate ratio ($P < 0.05$) similarly to butyrate and AGCR concentrations ($P < 0.05$). Chitosan increased milk yield ($P < 0.05$). Furthermore, dietary chitosan linear increased protein and lactose milk production ($P < 0.05$). Chitosan as feed additive interactions ($P > 0.05$) for any variable at any incubation time, excepting for the molar proportion of acetate at 24 h ($P = 0.019$). The method of processing the rumen contents did not affect ($P > 0.05$) either total VFA and CH$_4$ production or molar proportions of individual VFA at any time. At both incubation times, increasing the amount of concentrate in the substrate increased CH$_4$ production ($P < 0.001$, quadratic) and molar proportion of butyrate ($P < 0.001$, linear), but decreased acetate proportion ($P < 0.001$, quadratic) without affecting ($P > 0.05$) proportions of propionate. Whereas total VFA production was linearly decreased ($P = 0.007$) by increased amounts of concentrate in the substrate at 8 h of incubation, it was quadratically increased ($P < 0.001$) after 24 h of incubation. There were clear differences in CH$_4$ and VFA production among inocula from different sheep, which persisted across substrates. The results show that the tested methods of processing rumen contents did not affect in vitro fermentation characteristics of good quality substrates, but studies analyzing their possible influence on fermentation of low-quality substrates are required.

Key Words: Rumen content treatment, methane, volatile fatty acids

T488 Comparison of Roche 454 and Ion Torrent Personal Genome Machine sequencing on the rumen bacterial profiles of dairy cows. Nagaraj Indugu*, Sanjay Kumar, Bonnie Vecchiarelli, and Dipti Pitta, Department of Clinical Studies, School of Veterinary Medicine, New Bolton Center, University of Pennsylvania, Kennett Square, PA.

Next generation sequencing (NGS) is a widely accepted technology used by microbial ecologists for metagenomic analysis of complex microbial communities. As technologies continue to improve, it is necessary to compare data sets obtained from different platforms and analyze their effect on community structure. In the present study, we compared the 454 data set with that of Ion Torrent Personal Genome Machine (PGM) on the same DNA samples ($n = 14$) obtained from the rumen of dairy cows during their transition period. Despite the substantial difference in number of reads and length of reads, the platforms provided a similar community structure. The weighted UniFrac distances between the samples that were sequenced on both 454 and PGM were significantly correlated, as determined by Procrustes analysis of principal coordinate matrices ($M_2 = 0.319$; Monte Carlo $P < 0.001$). Though similar major abundant phyla were detected by both platforms, PGM recovered 4 additional phyla. At the genus level, there was no substantial variation between the 454 and PGM data sets for each animal except for Prevotella, Cyanobacteria YS2 and Succiniclasticum ($P < 0.05$; chi-squared test). However, there was variation in the abundance of genera between different animals, irrespective of the platform used. Collectively, the present study will be useful for microbiologists/ecologists to compare the microbial community structure obtained from different platforms; particularly with the expectation 454 will be completely replaced by PGM and/or Illumina.

Key Words: Ion Torrent PGM, microbial community, 454-Roche

T489 Effect of chitosan in dairy cows diets on ruminal fermentation and milk yield and composition. Carlos Eduardo Cardoso Consentini$^1$, Elmesome Ferreira de Jesus$^2$, Pablo Gomes de Paiva$^2$, Tiago Antonio Del Valle$^1$, Gustavo Ferreira de Almeida$^1$, Artur Gabriel Brao Vilas Boas Costa$^1$, Fernanda Carolina Ramos dos Santos$^1$, Victor Chiaromi Galvão$^1$, and Francisco Palma Remió$^1$, $^1$School of Veterinary Medicine and Animal Science of USP, Pirassununga, São Paulo, Brazil, $^2$School of Agricultural and Veterinary Sciences of UNESP, Jaboticabal, São Paulo, Brazil.

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