counts were done by surface-spread method and toxigenic ability of isolates strains was evaluated with in vitro conditions. Aflatoxins (AFs), ochratoxin A (OTA), fumonisin B1 (FB1) and deoxinivalenol (DON) natural contamination was determined with immunoaffinity columns extracts in HPLC. Total fungal counts were generally high (range 1.0×10^6 to 1.0×10^2 cfu g^{-1}). *Aspergillus flavus, Penicillium citrinum,* and *Fusarium verticillioides* were the prevalent toxigenic strains isolated. Mycotoxins levels differed ($P < 0.001$) from pre and post-fermentation samples, probably due to mold growth. Dry matter, carbohydrates, lipids, protein, volatile fatty acids, and fiber content not differed ($P < 0.001$) from pre and post-fermentation samples and were not different from literature. The inoculants does not helped in reducing the count of fungal propagules but decreased the biodiversity of the toxigenic strains presents in the treated silos. So, the use of microbial inoculant on silage production should be recommended to reduce some toxigenic strains contamination. However, each product must be evaluated and the applying process must be carefully conducted. The mycotoxin binding and nutritional quality increase related on literature was not observed on the present study.

Key Words: aflatoxin, corn silage, feedstuffs

T484 Utilization of equations to predict carbohydrate fractions in some tropical grasses. Romualdo S. Fukushima*, Carolina B. Bacha, Adriana P. Fuzeto, Ana C. R. Port, Valdo R. Herling, and Alejandro V. Velasquez, *Sao Paulo University, Pirassununga, SP, Brazil.*

The chemical composition of 5 tropical grasses, divided into stalk and leaf, at 3 maturity stages, was used to predict carbohydrate fractions by equations of the Cornell Net Carbohydrate and Protein System (CNCPS) or equations from our research group. Carbohydrate fraction A is a rapidly fermented pool that is primarily composed of sugars, some organic acids and short oligosaccharides. Fraction B1 is constituted mainly of starch and pectin. Fraction B2 has a slower rate of degradation and is available cell wall carbohydrates. The C pool is unavailable cell wall, which includes lignin. These carbohydrates fractions are estimated based on NDF analysis. However, NDF does not recover pectic substances and other ND soluble oligosaccharides such as β-glucans, fructans, galactans, etc. that are part of the cell wall matrix. Structurally, NDF is not complete plant cell wall. Then, a crude cell wall (CW) preparation, which represents plant cell wall matrix more completely because it preserves those carbohydrates that otherwise would be solubilized by the ND solution, was used in equations to predict carbohydrate fractions. By substituting NDF for CW, it was found that pectin and other ND soluble oligosaccharides (soluble fiber - SF) actually appeared in the fraction A of CNCPS, the rapidly degradable carbohydrate pool, instead of fraction B1, as proposed in the original CNCPS model. However, location of SF in the fraction A seems inadequate because degradation rate of SF is lower than fraction A components; thus, an alternative could be to place SF in a specific carbohydrate fraction (B2). This B2 fraction, soluble fiber, can be estimated by subtracting NDF from CW preparation. Because in the original CNCPS model the slowly degradable cell wall carbohydrates were assigned as fraction B2, we suggest naming this carbohydrate pool a new fraction, B3. With this arrangement, the fraction B1 would be constituted only by starch. These fractions are expressed on total carbohydrate basis, here suggested as: CHO = 100 - (CP + EE + MM + Lignin). This equation excludes lignin from the CHO compartment.

Key Words: cell wall, Cornell, soluble fiber

T485 Analysis of microbial populations in Rusitec fermenters fed diets of variable composition. Ivan Mateos[2], Maria Jose Ranilla*[2,3], Cristina Saro[2], Alexey Díaz[2], Maria Gracia De Garnica[2], Jairo Garcia[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.*

Fermenters are widely used to study ruminal fermentation, but information on microbial populations developing in fermenters over the incubation period is limited. Four Rusitec fermenters were fed 2 diets representative of those administered to dairy sheep (DAI; 50:50 alfalfa hay:concentrate) and fattening lambs (FAT; 15:85 barley straw:concentrate) in a crossover design with 2 14-d incubation periods to assess the evolution of the microbial populations. There were 4 fermenters per diet. The fermenters received daily 30 g of diet DM and samples from liquid (LIQ) and solid (SOL) digesta were taken on d 3, 8 and 14, and stored frozen at −80°C until DNA extraction. Concentrations of bacterial and protozoal DNA and relative abundance of fungi and methanogenic archaea to total bacterial DNA concentration were determined by real time PCR using previously validated primers and DNA from bacteria and protozoa isolated from sheep rumen as standards. Data were analyzed as a mixed model with repeated measures using the PROC MIXED of SAS. The model included diet, incubation run, time, and diet × time as fixed effects, and fermenter as a random effect. Diet x sampling time interactions ($P > 0.05$) were detected for bacterial and protozoal DNA concentrations in both digesta phases. The bacterial DNA concentrations in SOL did not change ($P = 0.002$) over the incubation period, whereas concentrations in LIQ increased ($P < 0.001$) by 1.5 and 1.8 times for DAI and FAT diets by the end of the incubation, respectively. Protozoal DNA concentrations on d 14 were 37.8 and 8.0 times lower ($P < 0.001$; means across diets) than those on d 3 for SOL and LIQ phases, respectively. Relative abundance of fungi decreased ($P < 0.05$) with time in both phases, and that of methanogenic archaea remain unchanged in LIQ and increased ($P = 0.021$) in SOL. Concentration of bacterial and protozoal DNA and the relative abundance of methanogenic archaea were greater in the fermenters fed the DAI diet ($P < 0.05$) compared with FAT diet. The results show that microbial populations in Rusitec fermenters are affected by the incubated diet and change over the incubation period.

Key Words: Rusitec fermenter, microbial populations, real-time PCR

T486 Influence of inoculum preparation method on in vitro methane production by ruminal microorganisms. Mireia Ramos[1], Ivan Mateos[2], Cristina Saro[2], Alexey Díaz[2], Maria Jose Ranilla*[2,3], 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.*

The characteristics of the inoculum are recognized as one of the most relevant factors influencing the results of in vitro fermentations in batch cultures of ruminal microorganisms. Four rumen-fistulated sheep fed a 66:34 alfalfa hay:concentrate diet were used as donors to investigate the effect of rumen contents' processing on in vitro methane (CH_4) and volatile fatty acid (VFA) production from 3 substrates of variable composition. Rumen contents were sampled from each individual sheep and subjected to the following treatments: SQ) squeezed through 4 layers of cheesecloth; FIL) SQ treatment and further filtration through a 100-μm nylon cloth; STO) treated with a Stomacher for 3 min at 230 rev min^{-1} and followed by SQ. The resulting fluids were used as inoculum for batch cultures containing alfalfa hay, concentrate, or a 50:50 mixture of both feeds. Cultures were incubated at 39°C for 8 and 24 h, and CH_4 and VFA production was measured. There were no treatment × substrate