

Rumen ciliates and methane production of beef bulls fed a biodiverse haylage, low-starch Mixed Diets

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This study evaluated the rumen ciliate protozoa (PTZ) and methane (CH₄) production of 20 crossbred young bulls (crossbred Charolais × crossbred Alentejana breeds) fed for 126 days with 4 total mixed diets including a legume-grass haylage as forage source and differing in forage:concentrate ratio (F:C 60:40 vs 75:25 (DM)), in the level of starch by replacing 50% of cereals in concentrate by low-starch agro-industrial by-products (LSBP) and in the addition of 6% (DM) of sunflower seeds (SS) as lipid supplement. Diets were: a) 60C: 60:40 for F:C, concentrate with cereals; b) 60S: 60:40 for F:C, concentrate with LSBP; c) 60SG: 60:40 to F:C, LSBP and SS; d) 75SG: 75:25 to F:C, LSBP and SS. Samples of rumen fluid were collected at slaughter and were incubated *in vitro* for 48h to estimate CH₄ production with the Ankom System. PTZ were evaluated by microscopic counting. ANOVA was used to evaluate the diet effects on total PTZ counts and individual genus proportions (% in total PTZ), total gas (Totgas) and CH₄ production (TotCH₄) and CH₄% (% of total gas). Relationships between PTZ, gas and CH₄ productions were evaluated by the Spearman's correlation coefficient. Diets did not affect total PTZ, but *Entodinium* was higher in bulls fed with 60C diet than with 60SG and 75SG ($P=0.011$) and *Epidinium* was lower with 60C than with 60SG and 75SG ($P=0.008$). Totgas and TotCH₄ productions were higher with 60C and 60S and lower with 75SG ($P=0.029$ and $P=0.048$, respectively). Total PTZ was not correlated with Totgas, TotCH₄ or CH₄%, but *Isotricha* ($P=0.047$) and *Eudiplodinium* ($P=0.030$) were correlate negatively with Totgas and *Epidinium* correlate negatively with Totgas ($P=0.010$), TotCH₄ ($P=0.002$) and CH₄% ($P=0.025$). *Entodinium* correlate positively with Totgas ($P=0.030$), TotCH₄ ($P=0.007$) and CH₄% ($P=0.004$). Conjugating LSBP with SS in the diets changed PTZ community composition and increasing haylage to 75% and supplementing with 6% SS reduced *in vitro* Gas and CH₄ productions. Rumen PTZ community was apparently related with gas and CH₄ determinations.

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