

Fungal pre-treatment increases *in vitro* rumen degradability of wheat straw

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Cellulose, the most abundant carbohydrate in the world, is an important nutrient in a ruminants diet. In plant cell walls cellulose, together with hemicellulose, is bound to lignin and forms the so called lignocellulosic complex. Lignin is difficult to degrade, and can only be degraded in an aerobic environment. The microbes in the rumen, an anaerobic environment, cannot degrade lignin, resulting in a low availability of cellulose as nutrient in highly lignified plant cell walls. Several methods are being used to degrade lignin and increase the nutritional value of lignocellulosic material. These chemical and mechanical methods are expensive and result in waste streams which can be harmful for the environment. A biological pre-treatment, using white rot fungi which can selectively degrade lignin without changing carbohydrate contents, can be a cheap and safe method. Previous studies showed increased *in vitro* rumen degradability of wheat straw after fungal treatment. However, the mechanisms behind this fungal treatment were not yet followed in detail. The aim of this experiment was to study fungal treatment more in detail, this should lead to a better understanding of the mechanisms behind it. Wheat straw was therefore inoculated with *Lentinula edodes* and *Ceriporiopsis subvermispora*. Every week, for 8 weeks, samples were taken and analyzed for *in vitro* gas production (IVGP) in rumen fluid. Selective lignin degradation was measured using the Van Soest (1991) method. Fungal biomass formed was determined by measuring ergosterol, a compound in the fungal cell.

L. edodes and *C. subvermispora* selectively degrade lignin without changing cellulose content. This process is reaching a plateau for *C. subvermispora* treatment after 5 weeks, while *L. edodes* continues lignin degradation until 8 weeks. Lignin degradation is positively correlated with IVGP, meaning that IVGP is increasing in time during the treatment. This also means that IVGP of wheat straw reaches a plateau after 5 weeks *C. subvermispora* treatment. Each fungus has thus a different strategy and degradation rate. Ergosterol measurements to determine growth confirm different strategies of the two fungi. *L. edodes* continues growth on wheat straw during 8 weeks, resulting in a good correlation ($R = 0.8$) between ergosterol and lignin degradation and IVGP. *C. subvermispora* colonizes the substrate within 1 week, and does not grow until 5 weeks of incubation after which growth continues. This fungus does not degrade lignin after 5 weeks of treatment, while absolute amounts of cellulose are decreasing from 6 weeks on. This suggests that during the second growth, starting at 5 weeks treatment, *C. subvermispora* uses cellulose to produce fungal biomass (correlation between fungal biomass formed and absolute cellulose amounts: $R = -0.81$ ($P < 0.001$)).

In conclusion, *L. edodes* and *C. subvermispora* do selectively degrade lignin in wheat straw and thereby increase IVGP. This process continues until 8 weeks for *L. edodes*, but reaches a plateau after 5 weeks treatment with *C. subvermispora*.