

Manganese and urea can increase lignin degradation by white rot fungi

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Introduction

Organic waste contains a considerable amount of cellulose, which can be used by rumen microbes as energy source. Lignin, present in the plant cell walls, blocks the cellulose and hemicellulose for degradation. The cellulose in plant cell walls is therefore not accessible for rumen microbes and in this way lignin is negatively related to digestibility. White rot fungi are specialists in degrading complex components such as lignin. In previous studies it was found that *Ceriporiopsis subvermispورا* and *Lentinula edodes* can degrade lignin selectively without degrading cellulose (Tuyen et al., 2012). Although fungal treatments can be very effective, it is a time consuming process. Additives can help the fungi to colonize the substrates faster or to stimulate enzyme production by the fungi to degrade more lignin. Adding nitrogen could enhance colonization of the substrate, however it is believed that lignin degradation in nature takes place in nitrogen poor environments (Tripathi and Yadav, 1992). In this study urea was tested as nitrogen source to see whether it can enhance the fungal treatment. Both *C. subvermispورا* and *L. edodes* do produce manganese peroxidase, an enzyme involved in lignin degradation. To do so manganese is needed, and using manganese as an additive it is expected that more lignin is degraded (Kerem and Hadar, 1995). Via this manganese pathway manganese peroxidase can only degrade phenolic lignin. To degrade non-phenolic lignin it is thought that manganese peroxidase is involved in a lipid oxidation pathway. From linoleic acid a lipid radical could be produced which can degrade non-phenolic lignin (Kapich et al., 1999).

The aim of this study was to see whether urea, manganese or linoleic acid added to fungal treatment using *C. subvermispورا* or *L. edodes* results in a faster increase in *in vitro* rumen degradability and lignin degradation. Wheat straw was used for the fungal treatment because it is a well described substrate in literature. Wood chips are a good substrate for a proof of principle because of the high lignin content.

Material and methods

Wheat straw and wood chips (oak) (particle size ~ 3 cm) were wetted until a final moisture content of 70% was reached. Additives were added to the substrates in two different concentrations: manganese (15 µg/g or 150 µg/g), urea (1 µg/g or 10 µg/g) or linoleic acid (0.5 mmol or 1 mmol). Substrates without additive were used as control to the additives. Both substrates were autoclaved for 1 hour at 121°C. *L. edodes* and *C. subvermispورا* were grown on malt extract agar, after which spawn was prepared on sorghum grains. Substrates were inoculated with the prepared grain spawn. Uninoculated substrates were used as control for fungal treatment. The experiment was performed in duplicate. After 4 weeks samples were taken and air-dried. *In vitro* gas production (IVGP) in rumen fluid was measured according to Cone et al. (1996). Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose (NDF-ADF) and cellulose (ADF-ADL) content were determined via the Van Soest method (Van Soest et al., 1991).

Results and discussion

L. edodes increased the IVGP of wheat straw with 56 ml/g OM to 270 ml/g OM (P<0.05). Wheat straw treated for 4 weeks with *C. subvermispورا* did not significantly increase the IVGP. Tuyen et al. (2012) found an increased IVGP from 3 weeks on with *L. edodes* and *C. subvermispورا*. A different batch of wheat straw used may explain the difference.

Manganese (15 µg/g) seemed to increase the IVGP, although not significantly. *C. subvermispora* degraded 30% more lignin when manganese (15 µg/g) was added than without any additive (P<0.05). Urea (1 µg/g) caused 16% more lignin degradation by *C. subvermispora* on wheat straw than without additive (P<0.05).

Additives did not have an effect on IVGP or lignin content in *L. edodes* treated wheat straw.

C. subvermispora increased the IVGP of wood chips by more than 200% (P<0.05). Additives did not have an additional effect on the IVGP or lignin degradation by *C. subvermispora* grown on wood chips.

Although *L. edodes* did not significantly increase the IVGP of wood chips, lignin degradation was enhanced by adding manganese (150 µg/g) to the culture.

Linoleic acid did not have an effect on fungal treatments by *L. edodes* or *C. subvermispora* on wheat straw or wood chips. This might be explained by the fact that manganese peroxidase is involved in the mechanism, and therefore manganese might be needed (Cunha et al., 2010). Using a combination of manganese and linoleic acid might stimulate fungal treatment.

Both fungi did not degrade cellulose of either wheat straw or wood chips, with or without additives. From this it can be concluded that the fungal treatment can increase the *in vitro* rumen degradability. More than four weeks are needed for this treatment to increase the *in vitro* rumen degradability. Improvement of the *in vitro* rumen degradability by the fungal treatment was not fastened by the additives tested here. Literature describes an additional effect of urea, manganese and linoleic acid. However in those studies different fungal species and/or substrates were used (Kapich et al., 1999; Cunha et al., 2010; Kerem and Hadar, 1995; Tripathi and Yadav, 1992). Nevertheless, although the IVGP was not increased, more lignin was degraded by *C. subvermispora* on wheat straw when manganese (15 µg/g) or urea (1 µg/g) were added. High variations were found within the duplicates, performing fungal treatment experiments in triplicate could give more clear answers.

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