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Effect of fungal treatments of fibrous agricultural by-products on chemical composition and *in vitro* rumen fermentation and methane production

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HIGHLIGHTS

- ▶ A fungus–substrate interaction was observed on nutritional values of fibrous by-products.
- ▶ Nutritional improvement by some fungi linearly increased with the level of lignin in substrates.
- ▶ *Pleurotus ostreatus* is an effective lignin degrader but could not improve the nutritive value of fibrous by-products.
- ▶ Treatment of oil palm fronds with *Ceriporiopsis subvermispora* reduced methane emission from rumen fermentation.

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ABSTRACT

Maize stover, rice straw, oil palm fronds and sugarcane bagasse were treated with the white-rot fungi *Ceriporiopsis subvermispora, Lentinula edodes, Pleurotus eryngii*, or *Pleurotus ostreatus* at 24 °C for 0–6 weeks. The fungi increased total gas production from oil palm fronds by 68–132%, but none of the fungi improved the *in vitro* rumen fermentability of maize stover. *C. subvermispora* and *L. edodes* increased total gas production of sugarcane bagasse by 65–71%, but *P. eryngii* and *P. ostreatus* decreased it by 22–50%. There was a linear relationship ($P < 0.05$) between the proportion of lignin in the original substrate and the increase in *in vitro* gas production observed for *C. subvermispora* and *L. edodes* treatments ($R^2 = 0.92$ and 0.96, respectively). It is concluded that *C. subvermispora* and *L. edodes* have a particularly high potential to improve the nutritive value of highly lignified ruminant feeds.

1. Introduction

Agricultural by-products such as rice straw, maize stover, oil palm fronds, and sugarcane bagasse, are abundantly available in many countries (Devendra, 2009; Sarnklong et al., 2010; Wan Zahari et al., 2003); however, their high neutral detergent fiber (NDF) and lignin contents, but low protein contents, cause low digestibility and feeding values for ruminants (Alborés et al., 2006; Islam et al., 2000; Karunanandaa and Varga, 1996; Karunanandaa et al., 1995; Wan Zahari et al., 2003).

Different technologies have been investigated to improve the feeding value of such by-products. Physical treatments, such as steaming, grinding and pelleting have been reported to increase the intake and digestibility of oil palm fronds and hence the performance of cattle (Wan Zahari et al., 2003). Alkali treatments, especially those with NaOH or NH_3, have been reported to improve the intake and/or digestibility of rice straw (Sarnklong et al., 2010), maize stover (Oliveros et al., 1993), oil palm fronds (Wan Zahari et al., 2003) and sugarcane bagasse (Amjed et al., 1992); however, such physical and chemical treatments can be expensive, harmful to users or environmentally unfriendly (Van Soest, 2006).

Biological methods, including the use of white-rot fungi and their enzyme extracts to improve the nutritive value of low quality feeds are regarded as environmentally friendly and potentially economically viable alternatives (Arora and Sharma, 2011; Arora et al., 2011; Bhuvnesh et al., 2011; Okano et al., 2006, 2007). Most of the data available in the literature where fungi have been used to improve the nutritional quality of potential feed ingredients, relate to the treatment of wheat straw using white-rot fungi (e.g. Agosin et al., 1985; Arora and Sharma, 2011; Arora et al., 2011; Bhuvnesh et al., 2011; Tuyen et al., 2012). To a lesser extent, data are available from work on rice straw, sugarcane bagasse and oil palm fronds (Karunanandaa and Varga, 1996; Karunanandaa et al., 1995; Okano et al., 2006, 2007; Rahman et al., 2011). The observed effects of the interaction of fungi with the substrates depend on the

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biochemical characteristics of the substrate, the used fungal strain and the length of the fungal fermentation (Alborés et al., 2006; Karunanandaa and Varga, 1996; Karunanandaa et al., 1995; Okano et al., 2006, 2007; Rahman et al., 2011; Sherief et al., 2010; Zadrazil and Puniya, 1995). There are no data available on the nutritive value of maize stover treated with fungi, although a high loss of lignin and other fiber components in maize stover treated with *Ceriporiopsis subvermispora* has been reported (Wan and Li, 2010).

Since the effect of fungi on the delignification and subsequent rumen degradation of a substrate is highly dependent on the substrate and fungal strain (Agosin et al., 1985; Akinfemi et al., 2010; Rahman et al., 2011; Tuyen et al., 2012), a direct implication of the results obtained from one substrate to other by-products may not be appropriate. Therefore, this study was conducted to identify the most promising strain among four white-rot fungi to improve the availability of nutrients in maize stover, rice straw, oil palm fronds and sugarcane bagasse to rumen microbes and to investigate the interaction between the fungal species and the substrate.

2. Methods

2.1. Fungal species and spawn preparation

C. subvermispora, *Lentinula edodes*, *Pleurotus eryngii* and *Pleurotus ostreatus* were selected for the current study as they had shown the highest potential in degrading lignin and improving the nutritive value of wheat straw (Tuyen et al., 2012). Cultivation and spawn preparation were carried out as previously described (Tuyen et al., 2012).

2.2. Preparation of substrate and method of cultivation

Rice straw and maize stover were collected after harvesting of the grains in Thailand and the Netherlands, respectively. The feedstuff was chopped by hand into pieces of 2–5 cm length and dried in an oven at 70 °C. Oil palm frond pellets and crushed sugarcane bagasse were obtained from Malaysia and Vietnam, respectively. Substrate sterilization and fungal inoculation processes were performed according to Tuyen et al. (2012). The inoculated substrates and the control (autoclaved but un-inoculated substrates) were incubated at 24 °C for 0, 21, and 42 days in an air-conditioned chamber with 75% relative humidity. For each substrate, three containers were prepared for each incubation period to represent three replications for chemical and statistical analyses. A sample in triplicate of each substrate was obtained before water was added to the dried substrates to determine the effect of autoclaving on chemical composition and total gas production.

2.3. Chemical analysis

Control and fermented substrates were dried immediately in an forced-air oven at 70 °C to constant weight to determine the dry matter (DM) content before being ground over a 1-mm screen using a Wiley hammer mill. Ash content was determined by combustion at 550 °C for 3 h in a muffle furnace. Ash-free NDF was analyzed by the modified method of Van Soest et al. (1991) with addition of a heat stable amylase. Ash-free acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed by the method of Van Soest (1973). The hemicellulose (HC) content was calculated as the difference between NDF and ADF, and cellulose (Cell) was calculated as the difference between ADF and ADL. Nitrogen content was measured by the Kjeldahl method (ISO 5983-1, 2005) and crude protein (CP) content was calculated as N × 6.25. The loss of DM and other nutrients due to the incubation with fungi was calculated from the difference, in absolute quantities, between the control and the fermented sample and expressed as a percentage of the total nutrient in the control.

2.4. In vitro gas and methane production

Gas production experiments were performed according to Cone et al. (1996). Rumen fluid was collected in the morning from two non-lactating Holstein–Friesian cows fed 1 kg of concentrate daily and grass silage *ad libitum*. Samples were incubated in triplicate with rumen fluid for 72 h to determine gas production characteristics of the control and the fermented substrates. Samples (10 µl) of gas, accumulating in the headspace of the gas production bottles, were collected at 0, 2, 4, 8, 12, 24, 36, 48, 56 and 72 h of incubation to determine the methane concentration in the fermentation gas, using gas chromatography (Perkin-Elmer GC8500) as described by Pellikaan et al. (2011).

2.5. Statistical analysis

Chemical composition, the 72 h accumulative gas production, and the losses of nutrients by the fungal incubation at 42 days compared to the control of the individual substrates were subject to generalized linear model (GLM) analysis in SAS 9.2 with the following model:

$$Y_{ij} = \mu + \alpha_i + \omega_{ij}$$

where Y_{ij} is the observation *j* in treatment (fungi) *i*; μ is the overall mean; α_i is the fixed effect of treatment *i*; ω_{ij} is the random error.

Post hoc multiple comparison with Tukey's significant test with $\alpha = 0.05$ was performed to determine the significance between the treatments. Regression equations to estimate the increase in total gas production (due to fungal treatment compared with the intact sample) from the content of lignin in the original substrate were established using the SAS 9.2 package.

3. Results and discussion

3.1. Changes in chemical composition

The autoclaving process affected the chemical composition of the substrates differently (Table 1). For maize stover and rice straw, autoclaving decreased the content of CP and ash and increased the content of NDF, ADF and ADL ($P < 0.05$). Autoclaving did not change the CP content of oil palm fronds and sugarcane bagasse as well as the ADF and ADL contents of the oil palm fronds ($P > 0.05$). The NDF content decreased but that of ADF and ADL increased in the autoclaved sugarcane bagasse compared to the original samples. These data confirm the results of Makkar and Sigh (1992) and Tuyen et al. (2012). The reason for the lower CP and ash content in the autoclaved substrate, compared to the original material, might be that steaming had vaporized or solubilized some nitrogenous compounds and other chemical elements which resulted in losses of N and ash and increases in the relative proportions of NDF, ADF and ADL components in the substrate.

There was a clear effect of the fungi on the chemical composition of the fermented samples. All fungi increased the CP content of the substrates after 6 weeks of incubation ($P < 0.05$). The proportion of CP increased because a portion of the carbohydrates was completely degraded to CO_2 and H_2O, causing a loss of OM but not N. Among the fungi, *P. ostreatus* treatment of maize stover, oil palm fronds and rice straw resulted in the highest and that of sugarcane bagasse in the lowest CP content. *P. eryngii* treatment, on the other hand, resulted in the lowest CP content in maize stover, oil palm fronds and rice straw, whilst the sugarcane bagasse treatment with *C. subvermispora* resulted in the highest CP content