Animal Nutrition xxx (2016) 1–9

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Optimizing bio-physical conditions and pre-treatment options for breaking lignin barrier of maize stover feed using white rot fungi

Andrew M. Atuhaire [a, b], Fred Kabi [c, *], Samuel Okello [b], Swidiq Mugerwa [a], Cyprian Ebong [d]

[a] National Livestock Resources Research Institute, UG-212 UG76 UG TR.TR., Tororo, Uganda
[b] Department of Livestock and Industrial Resources, School of Veterinary Medicine and Animal Resources, College of Veterinary Medicine, Animal Resources and Bio-security (CoVAB), Makerere University, UG-102 UG37 UG.KM.KM, Kampala, Uganda
[c] Department of Agricultural Production, School of Agricultural Sciences, College of Agricultural and Environmental Sciences (CAES), Makerere University, UG-102 UG37 UG.KM.KM., Kampala, Uganda
[d] Animal Production Program, Rwanda Agriculture Board (RAB), RW-01 RW12 RW.KV, Kigali, Rwanda

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form
5 August 2016
Accepted 17 August 2016
Available online xxx

Keywords:
Crop residues
Lactobacillus plantarum
Lignin biodegradation
Mycelial growth

ABSTRACT

The greatest limitation to utilization of maize stover by ruminants as a feed is the high concentration of lignin, which limits fibre digestibility. However, ruminants can effectively utilize maize stover if its nutritive value is improved using white rot fungal species. This study was designed to determine optimal bio-physical conditions for mycelial growth and select the most ideal fungal species and pre-treatment options for improving nutritive value of maize stover. Four popular edible *Pleurotus* fungal species (viz. *Pleurotus florida, Pleurotus ostreatus, Pleurotus sajor caju* and *Pleurotus pulmonarius*) were subjected to varying temperatures, pH levels, hydrogen peroxide (H_2O_2) concentration and illumination to establish the extent of mycelial growth rate. Inclusion of H_2O_2 was used to determine optimal levels for preservation and prevention of contamination from other indigenous microbiota. Effects of pre-treatment options on chemical composition and nutritive value of maize stover were also examined. Mycelial growth rate of *Pleurotus* species on potato dextrose agar (PDA) varied ($P < 0.05$) with temperature, pH level and H_2O_2 concentration following a quadratic trend. Optimal temperature, pH and H_2O_2 concentration for mycelial growth on PDA were 25 °C, 5 and 0.01 mL/L, respectively. Under the different bio-physical conditions, *P. sajor caju* had the highest mycelia density and growth rate. Chemical composition of solid-state fermented maize stover differed ($P < 0.05$) among the *Pleurotus* species. Maize stover fermented with *P. sajor caju* had the highest crude protein (CP) of 86.6 g/kg DM, *in-vitro* dry matter digestibility (IVDMD) of 731 g/kg DM, *in-vitro* organic matter digestibility (IVOMD) of 670.4 g/kg DM and metabolizable energy (ME) of 10.0 MJ/kg DM but with the lowest lignin (sa) of 50 g/kg DM. At 25 °C, *P. sajor caju* had the highest mycelial growth rate on PDA and highest lignin (sa) breakdown in the maize stover substrate. It was, therefore, selected as the most ideal fungal species for improving nutritive value of maize stover. Pre-treatment of maize stover with *Lactobacillus plantarum* and molasses under anaerobic condition for 7 days before inoculation with *P. sajor caju* resulted into a substrate with the highest ($P < 0.05$) CP (96.6 g/kg DM), IVDMD (752.3 g/kg DM), IVOMD (687.2 g/kg DM) and ME (10.2 MJ/kg DM). However, neutral detergent fiber exclusive of residual ash (NDFom) and lignin (sa) fractions decreased ($P < 0.05$) as a result of subjecting maize stover to pre-treatment with *L. plantarum* and molasses prior to fermentation with *P. sajor caju*. Therefore, pre-treatment of maize stover with

* Corresponding author.
E-mail address: fred.kabi@gmail.com (F. Kabi).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2016.08.009

Please cite this article in press as: Atuhaire AM, et al., Optimizing bio-physical conditions and pre-treatment options for breaking lignin barrier of maize stover feed using white rot fungi, Animal Nutrition (2016), http://dx.doi.org/10.1016/j.aninu.2016.08.009

L. plantarum and molasses for 7 days prior to fermentation with *P. sajor caju* for 14 days in darkness at 25 °C offered the greatest potential for breaking the lignin barrier.

1. Introduction

Although the history of lignin and cellulose chemistry is old with voluminous amounts of literature (Barton, 1988), the recalcitrant nature of lignin still limits the availability of nutrients to ruminants. This undermines the utilization of cereal stover and straws as ruminant feeds. Lignin is a phenolic compound of high molecular weight that adds rigidity to cell wall structure but limits the digestibility and availability of cell wall contents to rumen microbes (Chaudhry, 1998). Maize stover is rich in carbohydrates but its voluntary intake by ruminants is limited due to high levels of ligno-cellulosic bondages, which increase with plant maturity. Consequently, many farmers burn maize stover despite its potential as a source of energy for ruminants.

Although ruminants are known to have a highly effective digestive system for fibre, they are incapable of extracting sufficient energy and protein from maize stover to facilitate growth, production and reproduction (Montañez-Valdez et al., 2015). This is because the cell wall contents of maize stover, particularly cellulose and hemicelluloses, are locked into a complex polymeric compound, which is exquisitely constructed to resist biological and chemical hydrolysis (Mahesh and Mohini, 2013). Linkages between lignin with cellulose and hemicellulose inhibit accessibility of rumen microbial enzymes to the structural carbohydrates and, therefore, locking significant amounts of potential energy (Tengerdy and Szakacs, 2003).

Use of white rot fungi as a biological means to liberate carbohydrates from the ligno-cellulosic bondage has been widely reported as a promising, affordable and environmentally acceptable approach to increasing nutritional value of straws (Chaudhry, 1998; Tengerdy and Szakacs, 2003; Graminha et al., 2008). Furthermore, earlier studies indicated that pre-treatment of crop residues with a consortia of fungi working in a synergistic and syntropic association with other fungi, yeast such as *Saccharomyces serevisiae* or bacteria (*Lactobacillus plantarum*) would be preferable for better solid state fermentation (Wan and Li, 2012; Owen et al., 2012; Darwish et al., 2012). Darwish et al. (2012) indicated that maize stover treated with *Pleurotus ostreatus* and *S. serevisiae* at 28 °C for 7–28 days improved CP content from 36 to 118 g CP/kg DM. Meanwhile hemicellulose, cellulose and lignin were reported to decrease with increasing incubation time of fermentation process from 260.1 to 141.7 g/kg DM, 410.0 to 200.8 g/kg DM and 114.9 to 58.4 g/kg DM, respectively, when treated with a combination of white rot fungi and yeast. Similarly, Chen et al. (1995) observed that solid state fermentation of maize stover for 28 day at 27 °C with *Cyathus stercoreus*, *Phledia brevispora* and *Phanerochaete chrysosporium* increased *in-vitro* dry matter digestibility (IVDMD) from 409 g/kg (un-treated maize stover) to 514 g/kg DM (treated with *P. brevispora*) and 523 g/kg DM (treated with *C. stercoreus*) but growth of the white rot fungi *P. chrysosporium* resulted into reduced IVDMD from 409 to 298 g/kg DM. However, Fazaeli (2007) indicated that IVDMD of wheat straw treated with *Pleurotus florida* at 22 ± 5 °C for 17 days increased from 281 to 403 g/kg while when treated with *P. ostreatus* IVDMD increased from 281 to 370 g/kg DM. Both *P. florida* and *P. ostreatus* decreased lignin content of fermented wheat straw from 82 g/kg DM to 74 and 72 g/kg DM, respectively.

Ability of white rot *Pleurotus* fungal species to secrete exogenous enzymes that liberate and degrade lignin and yet preserve cellulose (Shirma and Arora, 2015) offers an attractive opportunity to harness the fungal species that secrete oxidative enzymes during mycelial colonisation (Dashtban et al., 2010). However, there is a paucity of information on the appropriate *Pleurotus* species, optimum bio-physical conditions and pre-treatment options that farmers in the tropics can use to improve the nutritive quality of fibrous crop residues including maize stover as livestock feed. The objectives of this study were, therefore, to: 1) determine bio-physical conditions that farmers can use to optimize mycelial growth rate and effectiveness of selected *Pleurotus* species, 2) identify the best *Pleurotus* species that can be used under these condition and 3) determine pre-treatment options that can improve the effectiveness of solid state fermentation of maize stover with the best *Pleurotus* species.

2. Materials and methods

2.1. Preparation of experimental fungal materials

The study was conducted in the animal science laboratory at the College of Agricultural and Environmental Sciences (CAES), Makerere University, Uganda. Pure stock commercial cultures of 4 white rot *Pleurotus* species (*Pleurotus* spp.) including *P. florida, P. ostreatus*, *P. sajor caju* and *P. pulmonarius* were procured from a Belgium based mushroom culture company (MYCELIA). The 4 fungal species are the most popular edible commercial mushrooms in Uganda. An agar block of pure stock culture per species was aseptically transferred to a sterilized 90 mm diameter petri dish containing sterile potato dextrose agar (PDA) (Formedium Hunstanton, England). The aseptic conditions of the petri dishes were achieved by sterilization at 121 °C for 15 min in an autoclave. Three petri dishes per fungal species were randomly inoculated with the pure stock culture and sealed with a parafilm to allow uniform mycelial ramification at 25 °C. After 7 days of fungal mycelial growth and ramification, a sterile cork borer was used to cut out round mycelial discs of 8 mm diameter. The discs were used as secondary mycelia of the pure cultures for each of the test *Pleurotus* spp.

2.2. Determination of fungal growth

The effects of varying temperature, pH, substrate-sterilization with H_2O_2 and illumination regimes on fungal mycelial growth of the four *Pleurotus* spp. were investigated using petri dishes containing sterile PDA substrates. The outside bottom of each petri dish was dissected into four quarters using vertical and horizontal axes and labeled as r1, r2, r3 and r4, respectively (Fig. 1). The secondary mycelial discs of the different *Pleurotus* spp. were transferred after 7 days of mycelial growth to the centre (intersection point) of the petri dishes. Mycelial growth was measured along each radius of the petri dish.

Please cite this article in press as: Atuhaire AM, et al., Optimizing bio-physical conditions and pre-treatment options for breaking lignin barrier of maize stover feed using white rot fungi, Animal Nutrition (2016), http://dx.doi.org/10.1016/j.aninu.2016.08.009