Effects of cocoa pod husk bioconversion with *Phanerochaete chrysosporium* and or *Pleurotus ostreatus* on its nutrient content and *in-vitro* digestibility in ruminants

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Abstract

This study was designed to investigate effects of bioconverting cocoa pod husk with *Phanerochaete chrysosporium* and or *Pleurotus ostreatus* on its nutrient contents and in-vitro digestibility in ruminants. Dried and ground cocoa pod husk was mixed with rice bran, CaCO3, and urea and sterilized by autoclaving. Sterilized cocoa pod husk was innoculated then with *P. chrysosporium* (0, 4, or 8 mg/kg) and or *P. ostreatus* (0, 15, or 30 mg/kg) in a 3 x 3 factorial experimental design resulting in nine treatment combinations, each of which was replicated three times. The fungi were allowed to ferment the cocoa pod husk and this was followed with proximate and fibre analyses on the cocoa pod husk to assess changes in its nutrients due to fermentation. An in-vitro digestibility test study was also done to investigate effects of the fermentation on the digestibility in ruminants digestive tracts.

Results indicated that the fungi did not change dry matter or organic matter contents of the cocoa pod husk. Either fungi increased crude protein content of the cocoa pod husk, and both microorganisms acted sinergetically to reduce that of crude fibre. An interaction effect was also found in the reduction of neutral detergent fibre and lignin content of the cocoa pod husk, but the highest reduction was found for the highest inclusion levels of the fungi in this study. Similarly, the highest combination levels of *P. chrysosporium* and *P. ostreatus* in fermenting cocoa pod husk tested in this study produced the highest in-vitro digestibility.

Keywords: *agricultural waste, fermentation, lignin, white rot fungi*

Introduction

Cocoa is an important plant in Indonesia, and the country is the second largest producer of cocoa beans in the world after Ivory Coast (FAOStat 2012). This indicates that a large quantity of cocoa pod husk is also produced in the country as a by-product. The total area of cocoa plantation in 2011 was about 1,68 million hectars (Agricultural Ministry 2012) which is equivalent to at least 4,54 million tonnes of dried cocoa pod husk.

Currently, cocoa pod husk produced from cocoa agriculture industry remains a waste. In fact, this biomass is a potential feed resource for ruminants which are equipped with rumen microorganisms capable of degrading coarse and fibre-containing substrates (Preston and Leng 1987). However, cocoa pod husk is characterized with high contents of crude fibre and lignin and low content of crude protein (Marsetyo 2008). These low nutritional properties of cocoa pod husk limit its utilization as an animal feed without appropriate treatments to increase its nutritional quality and digestibility in animals.

Phanerochaete chrysosporium and *Pleurotus ostreatus,* are two white rot fungi species that have gained increasing attention in biological treatment of organic materials containing high levels of crude fibre and lignin. These microorganisms produce ligninases which attack the lignin component of a substrate whereby reducing the molecular complex of ligno-cellulose. *P. chrysosporium* produces peroxidases, i.e. *lignin peroxidase* (LiP) and *manganese peroxidase* (MnP), while *P. ostreatus* produces *Laccase* in addition to the two peroxidases (Rothschild et al 1999).

While the two fungi have been used as biological agents to de-lignify a range of lignified substances separately, little is known on the lignifying effects of the two fungi when used simultaneously. In particular, no research has been done which use both the fungi simultaneously in bioconversion of agricultural by-products such as cocoa pod husk. This study was therefore designed to investigate effects of *P. chrysosporium* and *P. ostreatus*, alone or in combination, on changes in nutrient content of cocoa pod husk and its *in vitro* digestibility in ruminant animals.

Materials and Methods

Fermentation of cocoa pod husk

Dried and finely ground cocoa pod husk (10kg) was mixed with rice bran (1.5kg), $CaCO_3$ (0.15kg), urea (10g) and water (70% of the total weight) and mixed thoroughly. The mixture was put into polypropilene bags (1kg for each bag), sealed properly and the mixture-containing bags were sterilized by autoclaving at 121°C and 2 atm for 1h, and followed with cooling them at the room temperature for 24–36h. The sterilized cocoa pod was then innoculated with either *Phanerochaetechrysosporium* (10^7cells/g) and or *Pleurotus ostreatus* (14.3 x 10^9cfu/ml) according to the respective treatment level. The bags were placed in a room at a temperature of 25–30°C and air humidity of 80–90% to allow the fungi to grow.

When the cocoa pod mixtures inside the bag were completely covered with mycelium, the incubation was terminated and the bags were opened.

Experimental

The fermentation experiment was done in a 3x3 factorial design with *P. chrysosporium* level (0, 4, or 8 g/kg) as the first factor and *P. ostreatus* level (0, 15, or 30 g/kg) as the second factor. This resulted in the following nine treatment combinations, each of which was replicated three times:

Pc1Po1 = Cocoa pod without fermentation
Pc1Po2 = Cocoa pod + *P. ostreatus* (15g/kg)
Pc1Po3 = Cocoa pod + *P. ostreatus* (30g/kg)
Pc2Po1 = Cocoa pod + *P. chrysosporium* (4g/kg)
Pc2Po2 = Cocoa pod + *P. chrysosporium* (4g/kg) + *P. ostreatus* (15g/kg)
Pc2Po3 = Cocoa pod + *P. chrysosporium* (4g/kg) + *P. ostreatus* (30g/kg)
Pc3Po1 = Cocoa pod + *P. chrysosporium* (8g/kg)
Pc3Po2 = Cocoa pod + *P. chrysosporium* (8g/kg) + *P. ostreatus* (15g/kg)
Pc3Po3 = Cocoa pod + *P. chrysosporium* (8g/kg) + *P. ostreatus* (15g/kg)

Parameters observed were changes in dry matter, organic matter, crude protein, and crude fibre contents of the cocoa pod husk. Proximate analysis was done using standard procedures (AOAC 1990) while fibre component analysis (neutral detergent fibre, acid detergent fibre) was done according to Goering and van Soest (1987). Hemicellulose content was calculated as the difference between neutral detergent fibre contents with those of acid detergent fibre, while cellulose was taken as the acid detergent fibre minus lignin.

In vitro dry matter and organic matter digestibility of cocoa pod husk was carried out according to Tilley and Terry (1963) as modified by van der Meer (1980). Strained rumen fluid for the *in vitro* digestibility study was obtained from a rumen cannulated steer daily ration of 3 kg concentrate feed (a mixture of pollard, coconut meal, ground maize, premix, and mineral) and King grass ad libitum.

Results and Discussion

This study indicated that *Phanerochaete chrysosporium* or *Pleurotus ostreatus* did not affect dry matter or organic matter contents of cocoa pod husk. However, either *P. chrysosporium* or *P. ostreatus* increased the crude proteinconcentrations of the cocoa pod husk with the higher increase observed for higher levels of fungi application. On the other hand, a reduction was observed for crude fibre concentrations of cocoa pod husk after fermentation, and the two fungi acted sinergetically to affect this parameter.

Table 1: Dry matter, organic matter, crude protein and crude fibre contents of cocoa pod husk fermented with *Phanerochaete chrysosporium* (Pc) and or*Pleurotus ostreatus* (Po)

Nutrients (%)	***Phanerochaete chrysosporium***	***Pleourotus ostreatus***			**Means**	**SEM (p value)**
		Po1	**Po2**	**Po3**		
Dry matter	Pc1	88.8	87.4	87.3	87.9	

	Pc2	88.2	88.0	87.8	88.0	
	Pc3	88.1	87.8	87.5	87.8	
	Means	88.3	87.7	87.6		
Organic matter	Pc1	78.3	77.4	77.4	77.7	
	Pc2	77.9	76.7	76.6	77.1	
	Pc3	78.0	76.6	76.3	76.9	
	Means	78.1	76.9	76.8		
Crude protein	Pc1	6.87	7.32	8.10	7.43^{x}	
	Pc2	8.89	9.44	10.0	9.46^{y}	0.44(0.001)
	Pc3	9.20	10.4	10.6	10.1^{z}	
	Means	8.3^{A}	9.04^{B}	9.59^{C}		
	SEM (p value)		0.18(0.001)			
Crude fibre	Pc1	44.0^{a}	38.7^{b}	38.6^{b}	40.5^{x}	
	Pc2	38.3^{b}	36.1^{c}	35.0^{cd}	36.5^{y}	
	Pc3	36.8^{d}	33.7^{e}	33.1^{e}	34.4^{z}	
	Means	39.7^{A}	36.0^{B}	35.6^{B}		
	SEM (p value)		0.41 (0.007)			

-B) Different superscript letters indicate significant difference for P. chrysosporium
-e) Different superscript letters indicate significant difference for P. chrysosporium and P. ostreatus
-z) Different superscript letters indicate significant difference for P. ostreatus

Increased proportions of crude protein when fermented with *P. chrysosporium* or *P. ostreatus* is a common observation reported previously in some studies (Nelson and Suparjo 2011; Laconi 1998). However, such a phenomenon has to be interpreted cautiously as this not an indication of a nutritional improvement as a result of new true protein synthesis. In the current study, the increased crude protein concentrations was merely due to oxidation of dry matter during fermentation and to nitrogen contributed by the addition of rice bran (12% crude protein), urea and the fungi itself.

This study demonstrated that both fungi acted sinergetically in reducing the crude fiber contents of the cocoa pod husk (Table 1) and this can be attributed mainly to the reduction of neutral detergent fibre. It is known that both fungi produce fibre-degrading enzymes (Howard et al 2003) which degrade the fibre component of the fermented substrates. Reduction in lignin content of a substrate is the main feature of using *P. chrysosporium* or *P. ostreatus* a bioconverter of feed materials containing lignin. Van Soest (2006) stated that white rot fungi are effective agents to reduce the lignin content of a feed. Lignininase of *P. chrysosporium* has been described as an extracelullar enzyme which is secreted during nutrient starvation such as of nitrogen and carbon (Tien and Kirk 1988).

ble 2: Neutral detergent fibre, acid detergent fibre, cellulose, hemicellulose and lignin contents (%) of cocoa pod husk fermented with *Phanerochaete rysosporium* and or *Pleurotus ostreatus*

Fibre Components	***Phanerochaete chrysosporium***	***Pleurotus ostreatus***			**Means**	**SEM**
		Po1	**Po2**	**Po3**		**(p value)**
tral Detergent Fibre	Pc1	76.7^{a}	71.7^{b}	71.4^{b}	76.7^{x}	
	Pc2	71.7^{b}	70.9^{bc}	69.4^{d}	70.7^{y}	
	Pc3	69.4^{cd}	67.7^{e}	67.4^{e}	68.14^{z}	
	Means	72.6^{A}	70.1^{B}	69.4^{B}		
	SEM (p value)		0.54 (0.007)			
d Detergent Fibre	Pc1	57.2	55.9	55.8	56.3^{x}	0.89 (0.005)

	Pc2	56.4	55.6	54.9	55.6^{x}	
	Pc3	55.7	53.7	54.4	54.6^{y}	
	Means	56.4^{A}	55.1^{B}	55.0^{B}		
	SEM (p value)		0.27 (0.001)			
ulose	Pc1	33.1	32.6	32.2	32.6^{x}	
	Pc2	32.7	32.5	32.5	32.6^{x}	0.70 (0.025)
	Pc3	32.4	31.3	30.2	31.3^{y}	
	Means	32.8^{A}	31.9^{B}	31.7^{B}		
	SEM (p value)		0.19 (0.008)			
nicellulose	Pc1	19.5	15.8	15.6	17.0^{x}	
	Pc2	15.3	15.2	14.6	15.0^{y}	0.59 (0.044)
	Pc3	13.8	13.7	13.5	13.6^{z}	
	Means	16.2^{A}	14.9^{B}	14.5^{B}		
	SEM (p value)		0.26 (0.003)			
nin	Pc1	26.00^{a}	21.9^{b}	21.3^{b}	23.1^{x}	
	Pc2	21.4^{b}	20.4^{b}	19.2^{c}	20.4^{y}	
	Pc3	21.0^{b}	18.8^{cd}	18.7^{d}	19.5^{y}	
	Means	22.8^{A}	20.4^{B}	19.8^{B}		
	SEM (p value)		0.41 (0.008)			

Different superscript letters indicate significant difference for P. chrysosporium
Different superscript letters indicate significant difference for P. chrysosporium and P. ostreatus
Different superscript letters indicate significant difference for P. ostreatus

Reduction in fibre and lignin components of the fermented cocoa pod in this study was followed with an increase in in vitro dry matter or organic matter digestibility (Table 3). The highest digestibility was observed for the cocoa pod that had been fermented by both fungi indicating a greater accessibility for rumen microbes to degrade this substance compared to those were not fermented or fermented by individual fungus.

Table 3: In vitro dry matter and organic matter digestibility (%) of cocoa pod husk fermented with *Phanerochaete chrysosporium* and or *Pleurotus ostreatus*

Nutrient Digestibility	***Phanerochaete chrysosporium***	***Pleurotus ostreatus***			**Means**
		Po1	**Po2**	**Po3**	
Dry matter	Pc1	39.2^{a}	42.4^{b}	43.9^{c}	41.8^{x}
	Pc2	43.3^{bc}	46.0^{d}	48.0^{e}	45.8^{y}
	Pc3	47.3^{e}	48.3^{ef}	49.2^{f}	48.3^{z}
	Means	43.3^{A}	45.5^{B}	47.0^{C}	
	SEM (p value)		0.64 (0.008)		
Organic matter	Pc1	41.7^{a}	41.8^{a}	42.6^{ab}	42.0^{x}
	Pc2	42.0^{a}	43.4^{b}	46.2^{c}	43.9^{y}
	Pc3	45.9^{c}	48.3^{d}	48.4^{d}	47.5^{z}
	Means	43.1^{A}	44.5^{B}	45.8^{C}	
	SEM (p value)		0.53 (0.021)		

$^{(A-B)}$ *Different superscript letters indicate significant difference for P. chrysosporium*
$^{(a-e)}$ *Different superscript letters indicate significant difference for P. chrysosporium and P. ostreatus*
$^{(x-z)}$ *Different superscript letters indicate significant difference for P. ostreatus*

Conclusions

- *Phanerochaete chrysosporium* and *Pleurotus ostreatus*, alone or in combination, do not alter dry matter or organic matter content of cocoa pod husk after fermentation.

- *P. chrysosporium* and *P. ostreatus* interact one another in fermentation to reduce crude fibre, neutral detergent fibre and lignin content and increase in vitro digestibility of cocoa pod husk

- Higher effects were observed for higher inclusion levels of the microorganisms.

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