17.5%. Antiprotozoal effect of SRE and GE were confirmed in the present study. One possible mechanism to explain the inhibitory effect on protozoal growth is the change in the cell membrane permeability, as they form complexes with cholesterol in protozoal cell membranes and cause cell lysis (Hess *et al*., 2003). The structure and mechanism of action of garlic extract and its main active components on rumen microbial fermentaion are different from other compounds. Busquet *et al.* (2005) suggested that the antimethanogenic effect of garlic and its active components was the result of direct inhibition of Archaea microorganisms in the rumen. Archaea have unique membrane lipids that contain glycerol linked to long chain isoprenoid alcohols essential for the stability of the cell membrane (Kongmun *et al*., 2010). Goel *et al.* (2008) reported that *Sesbania saponins* decreased methanogen population by 78% and increased Fibrobacter *succinogenes* (21%-45%) and *Ruminococcus flavefaciens* (23%-40%). Pen *et al.* (2006), observed that the inclusion of *Quillaja saponaria* extraxt (QSE) resulted in decrease in protozoal population by 41%, but there was no effect on methane production. The other study informed the reduction of methanogens number by reduction of protozoa, as 10%-20% of total methanonogens reside in close assosiation with protozoa (Kumar *et al*., 2009). Ranilla *et al*. (2007) who conducted a study on the sheep rumen in *vitro* informed that the absence of ruminal protozoa in the rumen ecosystem decreased feed digestibility and methane proportion.

CONCLUSION

The supplementation of 1.8 g *S. rarak* extract and 0.25 ppm garlic extract per kilogram ration represents the best combination for dairy cattle feed containing adeguate Cr, Se, and Zn minerals to improve ruminal fermentation based on feed digestibility, fermentation products, and rumen bacterial population.

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Nutritive Value of Coffee Husk Fermented with *Pleurotus ostreatus* as Ruminant Feed

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ABSTRACT

Coffee husks is an abundant crop residue but the content of anti nutritional substances such as caffeine, tannin, and lignin limit its utilization as feed ingredients. Higher fungi such as *Pleurotus ostreatus* have the ability to biotransform lignocellulosic materials through their extracellular enzyme activities. This study was carried out to assess the effect of solid state fermentation by using *P. ostreatus* on nutrient composition of coffee husk and to evaluate its potency as ruminant feed *in vitro*. The *in vitro* experiment was conducted to determine fermentability of treated coffee husk. The usage rate of fermented coffee husk was mimicked feeding level to mid lactation dairy cows; 0%, 10%, 20%, 30%, and 40% (R0 to R4). Fermentation of coffee husk by *P. ostreatus* increased its protein, from 10.36% to 12.14%, and cellulose, from 19.51% to 24.80%, and decreased its lignin, from 65.42% to 45.04%, tannin from 1.02% to 0.18%, and caffeine, from 1.39% to 0.20%, concentrations. There were no differences in ruminal pH and N-ammonia production but volatile fatty acid production and dry matter digestibility decreased as the fermented coffee husk level increased. The ruminal protozoa population in fermented coffee husk diets was lower than the control diets (P<0.05). In conclusion, it is possible to use 20% of fermented coffee husk in the ration.

Key words: coffee husk, Pleurotus ostreatus, nutritive value, ruminant feed

ABSTRAK

Kulit buah kopi merupakan suatu limbah pertanian yang cukup banyak ketersediaannya, tetapi bahan-bahan anti nutrisi yang dikandungnya seperti kafein, tannin dan lignin membatasi penggunaannya sebagai bahan pakan. Jamur kelas tinggi seperti *Pleurotus ostreatus* memiliki kemampuan untuk mengubah secara biologis bahan-bahan lignoselulosa melalui aktivitas enzim ekstraseluler yang dihasilkannya. Penelitian ini bertujuan untuk mengevaluasi komposisi nutrisi kulit buah kopi yang difermentasi dengan *P. ostreatus* dan potensi kulit buah kopi hasil fermentasi sebagai bahan pakan ruminansia secara *in vitro*. Percobaan pemberian pakan *in vitro* bertujuan untuk menentukan fermentabilitas kulit buah kopi yang telah difermentasi. Tingkat penggunaan kulit buah kopi yang telah difermentasi disusun untuk memenuhi kebutuhan nutrisi sapi perah yang berada pada fase laktasi pertengahan; 0%, 10%, 20%, 30% dan 40% (R0 sampai R4). Hasil penelitian menunjukkan bahwa terdapat peningkatan kadar protein dari 10,36% menjadi 12,14% dan kadar selulosa dari 19,51% menjadi 24,80%, dan penurunan kandungan lignin dari 65,42% menjadi 45,04%, tanin dari 1,02% menjadi 0,18%, dan kafein dari 1,39% menjadi 0,20%. Tidak terdapat perbedaan dalam pH rumen dan produksi N-amonia tetapi produksi *volatile fatty acid* (VFA) dan kecernaan bahan kering menurun seiring dengan meningkatnya level kulit buah kopi fermentasi. Jumlah populasi protozoa dalam ransum yang mengandung kulit buah kopi fermentasi menurun dibandingkan ransum kontrol (P<0,05). Dapat disimpulkan bahwa pemanfaatan kulit buah kopi hasil fermentasi sampai 20% memungkinkan untuk diterapkan.

Kata kunci: kulit buah kopi, Pleurotus ostreatus, kualitas nutrisi, pakan ruminan

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INTRODUCTION

Coffee is an agricultural crop of significant economic importance in Indonesia. The coffee fruits are generally processed through the dry method and a little part processed through the wet method, both yielding by product (coffee husk and coffee hull). At present, large quantities of by-products are accumulated at production site. Traditionally, they are removed to be burned or end up in the river and small amount returned into the field as fertilizer. Therefore, this has led the problem of environment pollution.

Coffee husk is obtained after drying and de-hulling coffee fruits in dry process method. Coffee husks are available in large quantities, accounting for about 21.5% from total weight of the coffee fruits (Muryanto *et al*., 2005). Coffee husks are barely utilized in animal nutrition because of anti-nutrient substances such as caffeine, tannins, lignin and other polyphenols (Orozco *et al*., 2008). The presence of tannins and caffeine diminish acceptability and palatability of coffee husk by the animal (Mazzafera, 2002).

The coffee husk has potency as a source of ruminant feed. The protein content is 9.2%-11.3% (Fan & Soccol, 2005). Its cell wall fractions can be utilized by ruminant as a source of energy (Russel *et al*., 2009). However high lignin content limits digestibility of cellulose and hemicellulose.

Application of biotechnology is worth considering for the improvement of nutritional value of crop residues. Coffee husk is rich organic content, and is a suitable substrate for fermentation processes. Higher fungi or mushrooms have the ability to biotransformed fibrous agro-residues into value added product through their extracellular enzymes activities. *P. ostreatus* is one of the popular cultivated mushroom. It can be cultivated on a wide range of lignocellulosic substrates such as wheat straw, sugar cane bagasse and cocoa husk (Fazaeli *et al*., 2004; Okano *et al*., 2007; Alemawor, 2009). *P. ostreatus* belongs to white rot fungi which are able to degrade lignin because produce ligninolytic extracellular enzymes, such as laccase, lignin peroxidase and Mn peroxidase (Mayer & Staples, 2002; Periasamy & Natarajan, 2004).

The ability of *P. ostreatus* degrades a wide variety of lignocellulosic substrates, enabling it to play an important role in managing organic wastes whose disposal is problematic. *Pleurotus* species have been used by human for their nutritional value, medicinal properties, transformation of wastes into animal feed and other beneficial effects (Gregori *et al*., 2007; Ijeh *et al*. 2009; Darwish *et al*., 2012). This study was carried out to determine the effect of a solid state fermentation involving *P. ostreatus* on nutrition composition and nutritive value of coffee husk.

MATERIALS AND METHODS

Coffee husks were obtained from coffee hulling plant at Rejang Lebong Residence Bengkulu Province, one of the major coffee producers in Sumatera Island. Coffee husks were air-dried to moisture content of 10%-15% and then fermented with *P. ostreatus*. The solid-state fermentation trials were carried out on a laboratory scale. The solid state substrate was prepared with the composition adopted from sawdust standard substrate (Herliyana *et al*., 2008). The substrate were consisted of 82.5% coffee husk, 15% rice bran, 1.5% gips, and 1.0% $CaCO_3$. The clean water was added to the substrate as much as 65%-70% (v/w). These components were composted for 24 h and then were placed in polypropylene bag in amount 400 g per bag. Each bag was closed with a small cotton plug inserted in the middle of its opening. The bags were sterilized at 121 ^oC for 30 min. Each bag was seeded with ±15 g (3.75%) of *P. ostreatus* spawn. All spawned bags were placed in a growing room with the temperature of 22-28 ^oC and relative humidity of 60%-80% for a 60-d incubation period.

The fully colonized substrate or bag logs were opened and prepared for analysis. The substrate was dried in an oven at 60 ^oC for 2 d and then ground. The non fermented coffee husk and the fermented coffee husk were sampled for nutrient composition according to proximate analysis. The cell wall fractionations (neutral detergent fiber/NDF, acid detergent fiber/ADF, lignin, cellulose, and hemicellulose) were carried out according to the method as described by Goering & Van Soest (1970). Tannin was determined using Folin Ciocalteau (Harborne, 1987). Caffeine was analyzed according to AOAC Official Method (2005).

The fermentation characteristics (ruminal pH, NH_3-N and volatile fatty acid/ VFA concentration), the rumen protozoa population, and *in vitro* digestibility were analyzed to evaluate its nutritive value. The ruminal pH was measured using pH-meter. Ammonia-N concentration analyzed by Conway microdifusion method. VFA were analyzed by steam distilation method. *In vitro* digestibility was evaluated according to Tilley & Terry method (1963).

The rumen protozoa population measurement was done on counting chamber. As much as 0.5 ml rumen solution was fixed with 0.5 ml saline solution (Methylgreen Formaline Saline/MFS) in tubes and well mixed (Ogimoto & Imai, 1981). The sample as much as 0.1 ml of was dripped by the pipette on counting chamber (hemacytometer) and covered with covered glass. The protozoa were counted on the counter under a microscope using 40x magnification. From the number of protozoa obtained from the counting procedure above, the number per 1 mL of rumen contents can be calculated by following formula:

Protozoa population/mL= (1 / 0.1 x 0.065 x 5 x 16) x n x d

where: n= number of protozoa on the counting chamber
d= diluted multiple of the sample.

Up to 40% of fermented coffee husk with 10%-increment were tested (Table 1). Dietary samples were formulated as diets recommended for midlactation dairy cows (TDN ≤68% and crude protein 11%-13%) (NRC, 2001). A randomized block design was used to allocate three rumen fluids as block, five dietary samples composed of different fermented coffee husk levels in two replicates. Incubation was conducted for 48 h for NH_3-N and VFA analysis, while 48 h incubation for dry matter digestibil-

Table 1. Ingredients, composition, and nutrient contents of diets (%)

Ingredients	R0	R1	R2	R3	R4
Elephant grass	60	50	40	30	20
Fermented coffee husk	0	10	20	30	40
Coconut meal	5	0	0	0	0
Cassava waste	15	13	12	12	10
Pollard	5	8	8	8	10
Soybean meal	6	6	5	5	5
Rice bran	8	12	13	14	14
Lime stone	1	1	1	1	1
Crude protein	13.32	13.23	13.03	13.06	13.42
TDN	61.00	61.70	62.84	64.24	65.46

Notes: The level of fermented coffee husk in the diet: R0 (0%/control), R1 (10%), R2 (20%), R3 (30%), R4 (40%).

ity evaluation. Data were subjected to one-way analyses of variance (Steel & Torrie, 2003).

RESULTS AND DISCUSSION

The Nutrient Composition

The changes in nutrient composition during the *P. ostreatus* mycelia growth period are shown in Table 2. There were decreases in fiber fraction (lignin, NDF, and ADF) upon biofermentation (16.60%, 15.00% and 31.20% decreases in NDF, ADF, and lignin respectively as compared to non fermented ones).

The cellulose, hemicelluloses, and lignin are the main sources of carbon and energy for *P. ostreatus* growth, while protein serves as the N source. The decrease in fiber fraction results from cell wall degradation ability of *P. ostreatus*. Similar reduction in NDF and ADF contents of fungi treated coffee by product has also been reported by Penaloza *et al.* (1985).

Table 2. Changes of nutrien contents of coffee husk substrate fermented by *Pleurotus ostreatus* before and after fermentation (as % dry matter)

Nutrient component	0 day (Before fermentation)	After fermentation	% Changes
Organic matter	93.71	86.6	-7.59
Crude protein	10.36	12.14	17.2
Neutral detergent fiber	95.18	79.08	-16.6
Acid detergent fiber	87.18	74.08	-15
Hemicellulose	7.99	5.32	-33.41
Cellulose	19.51	24.8	27.11
Lignin	65.42	45.04	-31.12
Tannin	1.02	0.18	-82.35
Caffeine	1.39	0.2	-85.61

Decreased lignin content upon fungi treatment is important for ruminant nutrition. *Pleurotus spp*. releases ligninolytic enzyme that degrade lignin (Fan *et al*., 2008; Widiastuti *et al*., 2008). Platt & Hadar (1983) noted that during the mycelia growth period, *P. ostreatus* mycelia were more capable to degrade lignin, and the degradation of lignin played an important role in mycelia development.

The decrease of lignin concentration in this research (31.20%) was lower than rice straw and wheat straw fermented by *P. ostreatus* that were 46.18% for rice straw (Jafari *et al*., 2007) and 53.76% for wheat straw (Patil *et al*., 2010). This condition showed that there were difference of ligninolitic charateristics among substrate which might be caused by the difference substrate form and the initial lignin concentration. The initial lignin concentration of rice straw (9.55%) and wheat straw (24.18%) were lower than coffee husk (65.42%).

Upon fermentation the rate of decrease in hemicellulose concentration (-33.42%) may suggest that hemicellulose is more easily degraded than cellulose and lignin. Hemicellulose is more easily degraded than cellulose and lignin (Perez *et al*., 2002). *P. ostreatus* needs a carbon source which is easier to metabolize. Hemicellulose degradation is required before efficient lignin removal can commence (Sanchez, 2009).

The cellulose content increased 27.11%. Biofermentation broke the lignocelluloses bond and gained cellulose. Delignification has important role in mycelia growth which cleavage polysaccharide component (cellulose and hemicellulose). This component is utilized by fungi as substrate for their growth (Baldrian & Gabriel, 2003).

The protein content increased to 17.20% after fermentation. This increase in protein content could be attributed to possible formed fungal biomass. Fungal cell in mycelia contributed the protein content of substrate. Sixty and 70% of N present in the fungal cell is protein (Chang & Miles, 2004). The higher protein content in the substrate was prepared to transferable nitrogen into fruit bodies of mushroom. The extensive formation of primordia indicated the end of the vegetative growth phase of *P. ostreatus.* As coffee husks substrate was degraded and its nutrient used by *P. ostreatus*, the total organic matter of substrate decreased (Table 2).

The content of caffeine and tannin are of interest in terms of the potential use of coffee husk as animal feed. Tannin adversely affects feed digestibility and N utilization by the animals (Makkar, 2003). The caffeine concentration was reduced to 85.61% and to 82.35% for tannin after fermentation (Table 2). It means that Pleurotus was capable of degrading phenolic compound present in the coffee husk. The reduction of tannin in this study (82.35%) was also supported by Fan *et al*. (2006) who reported that there was decrease in tannin as much as 79.1% in coffee husk after cultivation

The tannin degrading ability of Pleurotus on coffee husk (82.35%) was better than other edible mushrooom *Flammulina velutipes* that was decrease 20.4% (Fan *et al*., 2001). This variation was affected by the fungi used might be attributed to strain differences, length of fer-

Table 3. The ruminal pH, N-NH_3, VFA, protozoa number, and dry matter digestibility produced from diets supplemented with bio-conversion product

Parameter	R0	R1	R2	R3	R4
pH	6.95± 0.01	6.91± 0.07	7.05± 0.03	7.06± 0.15	6.99± 0.061
VFA (mmol/L)	158.30±31.7^a	125.00±16.4^b	121.20±11.0^b	117.70±12.9^b	107.50±13.0^b
N-ammonia (mM)	12.94± 4.80	11.84± 5.90	12.14± 5.94	13.41± 5.80	12.23± 7.35
Protozoa, cell/mL (Log10)	6.45± 0.10^a	4.95± 0.22^b	4.76± 0.00^b	4.89± 0.06^b	5.00± 0.23^b
Dry matter digestibility (%)	66.80± 1.57^a	59.19± 2.35^b	56.22± 1.89^b	52.21± 0.53^c	47.51± 0.38^d

Notes: Means in the same row with different superscript differ significantly (P<0.05). Level of fermented coffee husk in the diet: R0 (0%/control), R1 (10%), R2 (20%), R3 (30%), R4 (40%).

mentation, and the physiological behaviour difference (Akinfemi & Ogunwale, 2012).

The reduction of caffeine and condensed tannin in *Pleurotus* treated agreed with trends found by Rojas *et al.* (2002) who used a microorganism as biological inoculants to treat the wet coffee pulp. Fan *et al.* (2006) also reported the reduction of caffeine and tannin in coffee husk after cultivation of *Pleurotus.* They also found that coffee husk was an excellent substrate for mushroom cultivation, especially *P. ostreatus. Pleurotus* species was able to detox the coffee husk.

The increasing protein and cellulose contents and the decreasing lignin and anti-nutritional substances (tannin and caffeine) in the coffee husk after fermentation by *P. ostreatus* can increase its value as by-product in ruminant nutrition.

In Vitro Fermentation

Table 3 shows the fermentability (ruminal pH, NH_3-N, and VFA production), ruminal protozoa population, and dry matter (DM) digestibility of fermented coffee husk. No statistically significant differences were observed in ruminal pH (P>0.05). The mean ruminal pH in this study were in normal range as Sung *et al.* (2007) reported that the ideal ruminal pH for keeping normal rumen metabolism was 6.0-7.0. Fiber digestion decreases at low rumen pH, especially below pH 6.0.

Increasing fermented coffee husk up to 40% decreased total VFA concentration from 158.3 to 107.5 mmol L^{-1} (P<0.05). This was probably due to still high lignin. The structural carbohydrate (cellulose and hemicellulose) in fermented coffee husk which was used to 40% in the diets could not be degraded well by rumen microbes. This result was also supported by Xu *et al.* (2007) who reported that ruminal fluid from Holstein steers or sheep receiving coffee ground had significantly lower concentration of total VFA than those receiving no coffee ground. No alteration in NH_3-N level (11.84-13.81 mM) in response to increasing fermented coffee husk level may indicate that inclusion fermented coffee husk to 40% in diets does not interfere with ruminal protein metabolism.

The ruminal protozoa population in control diets was higher than the fermented coffee husk diets (P<0.05). No statistically difference were observed in ruminal protozoa among fermented coffee husk diets. This condition indicated that the bioconversion product contained antimicrobial compounds.

Macrofungi need antibacterial compounds to survive in their natural environment. Macrofungi produced secondary metabolites, such as phenols, flavonoids, tannin and terpenoids compounds which presented antimicrobial activity (Lindequist *et al*., 2005; Patel *et al*., 2012). Macrofungi possessed the primary metabolites, such as polysaccharides, oligosaccharides, protein, and conjugated compounds such as glicoprotein, lipoprotein, proteoglican that formed an integral part of the fungal cell wall. These bioactive compound also exhibited antimicrobial properties (Stamets, 2002; Iwalokun *et al*., 2007; Chan *et al*., 2009).

The inclusion of fermented coffee husk in the diets decreased the dry matter digestibility (P<0.05). The inclusion more than 20% fermented coffee husk in the diets had DM digestibility lower than 55%, the value of which lower than the minimum recommended degradability (55%) for poor quality tropical roughages (Preston & Leng, 1987). This result was supported on other studies with calves and dairy heifers which had drastic reduction of feed intake and lower nitrogen retention when coffee pulp exceeded 20% in ration (Cabezas *et al.,* 1987).

CONCLUSION

The treatment of coffee husk with *P. ostreatus* can increase protein and cellulose concentration, but decrease lignin, tannin, and caffeine concentration. There are no differences in ruminal pH and N-ammonia production. The fermented coffee husk diets decrease the ruminal protozoa. Volatile fatty acid and dry matter digestibility decreased as the level of fermented coffee husk increased.

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