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Fermentation Technology using *Phanerochaete chrysosporium* to Improve the Quality of Nutrition of Pod Coffee as Ruminant Feed

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ABSTRACT

This study was carried out to assess the effect of solid state fermentation by using *P. chrysosporium* on nutrient composition of pod coffee and to evaluate its potency as ruminant feed *in vitro*. The *in vitro* experiment was conducted to determine fermentability of treated pod coffee. Fermented pod coffee by *P. chrysosporium* 0, 1, 2, 3, 4 % (R0 to R4). Pod coffee were air dried to moisture content of 10%-15% and then fermented with *P. chrysosporium*. The solid state fermentation trials were carried out on a laboratory scale. The results of this study were fermentation of pod coffee by *P. chrysosporium* increased protein from 10.36% to 12.64%, and cellulose from 18.51% to 23.80%, and decreased lignin from 64.42% to 44.04%, tannin from 1.02% to 0.18%, and caffeine from 1.39% to 0.20%. There were no differences in ruminal pH and N-ammonia production, but volatile fatty acid production and dry matter digestibility decreased as the fermented of pod coffee level increased. The ruminal protozoa population in fermented of pod coffee diets was lower than the control diets ($P<0.05$). Conclusion of this study is that fermentation of pod coffee with *P. chrysosporium* can increase protein and cellulose concentration, but decrease lignin, tannin, and caffeine concentration.

Key words: Pod coffee, *Phanerochaete chrysosporium*, Nutritive value, Ruminant feed

Introduction

Indonesia is the third largest coffee producer in the world after Brazil and Vietnam, by contributing about 6% of the world's total coffee production, and Indonesia is the world's fourth largest coffee exporter by share market about 11% in the world. Pod Coffee is potential enough to be used as feed material for ruminants, both small ruminants and large ruminants. The nutritional content of non-fermented pod coffee such as crude protein is 8.49%, is relatively comparable to the nutritional content of grass. Pod coffee is given directly in the wet form, the water content is high enough so that it is easily damaged and less preferred by livestock. But apart from that it's high crude fiber content and the presence of tannins, caffeine and lignin in non-fermented pod coffee which can interfere with livestock digestion if given in large quantities. One of the ways to minimize these limiting factors, the pod coffee is processed first before being given to livestock. One of the processing processes that can be done is fermentation technology.

Fermentation is one of the technologies to change feed to increase its nutritional content (protein and energy) and is preferred by livestock because of the fragrant aroma of fermentation. Improving the quality of nutrition in pod coffee through particle reduction and fermentation can significantly increase crude protein, lower crude fiber and TDN (Mazzafera, 2002). The benefits of fermentation are to increase digestibility and palatability, increase protein content, reduce crude fiber content and lowering the tannin content. One that affects consumption is the palatability (level of preference for livestock) of the type of food given (Charray *et al.*, 1992). Livestock greatly like the fermentation of pod coffee this may be due to the aroma of fermentation that cattle like. Morand-fehr (2003) states that important factors come from food that affects consumption is the aroma of the food material itself, livestock can refuse food that is given without tasting it first, because they do not like the aroma.

Pod coffee is obtained after drying and de-hulling coffee in dry process method. Pod coffee are available in large quantities, accounting for about 21.5% from total weight of the coffee fruits (Muryanto *et al*., 2009). Pod coffee 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).

Pod coffee has potency as a source of ruminant feed. The protein content is 9.2%-11.3% (Fan and 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. Pod coffee 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. In addition to lignin*, P. chrysosporium* completely degrades all major components of plant cell walls including cellulose and hemicellulose (Martinez *et al.,* 2004). *P. chrysosporium* maximum degradation of caffeine and tannins were 70.8 and 45%, respectively, with coffee husk having 65% moisture and pH 5.5 in 14 days (Brand *et al.,* 2000).

The ability of *P. chrysosporium* degrades a wide variety of lignocellulosic substrates, enabling it to play an important role in managing organic wastes whose disposal is problematic. This study was carried out to determine the effect of a solid state fermentation involving *P. Chrysosporium* on nutrition composition and nutritive value of coffee husk.

Materials and Methods

Pod coffee were obtained from coffee hulling plant at Wonogiri residence, Central Java Province. Pod coffee were air dried to moisture content of 10%-15% and then fermented with *P. chrysosporium*. 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% pod coffee, 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. chrysosporium* 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^o C for 2 d and then ground. The non fermented pod coffee and the fermented pod coffee 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 and Van Soest (1970). Tannin was determined using Folin Ciocalteau (Harborne, 1989). Caffeine was analyzed according to AOAC (2005) official method.

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 and 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 and 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.

Table 1. Analysis proximat pod coffee (% dry matter)

Nutrient	%
NDF	82.34
ADF	84.25
Lignin	47
Cellulose	63
Hemicellulose	2.3
Organic Matter	91.23
Crude protein	115
Crude fiber	32.15
Crude fat	0.26
Tannin	1.8
Caffeine	1.28

The treatment usage pod coffee fermented by *P. chrisosporium* using four treatment with five replication. The treatments were R0= cocoa pod fermentation without mold adding, R1 = pod coffee fermented with *P. chrysosporium* 1%, R= pod coffee fermented with *P. chrysosporium* 2%, R3= pod coffee fermented with *P. chrysosporium* 3% and R4 pod coffee fermented with *P. chrysosporium* 4% . Incubation was conducted for 48 h for NH_3-N and VFA analysis, while 48 h incubation for dry matter digestibility evaluation. Data were subjected to one-way analyses of variance (Steel and Torrie, 2003).

Results and Discussion

The nutrient composition

The changes in nutrient composition during the *P. chrysosporium* mycelia growth period are shown in Table 2. There were decreases in fiber fraction (lignin, NDF, and ADF) upon biofermentation (31.12%, 16.60%, 15.00% and 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. chrysosporium* growth, while protein serves as the N source. The decrease in fiber fraction results from cell wall degradation ability of *P. chrysosporium*. Similar reduction in NDF and ADF contents of fungi treated coffe by product has also been reported by Penaloza *et al.* (1985).

Decreased lignin content upon fungi treatment is important for ruminant nutrition. The decrease of lignin concentration in this research (31.12%) was lower than rice straw and wheat straw fermented by *P. chrysosporium* 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 pod coffee (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. chrysosporium* needs a carbon source which is easier to metabolize. Hemicellulose degradation is required before efficient lignin removal can commence (Sanchez, 2009).

The results showed that the best cocoa pod fermentation by the addition of *Phanerochaete chrysosporium* produced LiP enzyme activity and the highest MnP 0.17±0.001 U/ml and 0.09±0.001 U/ml (Yakin et al., 2017). 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 and 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 and 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. chrysosporium.* As pod coffee substrate was degraded and its nutrient used by *P. chrysosporium*, the total organic matter of substrate decreased.

The content of tannin are of interest in terms of the potential use of pod coffee 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 *P. chrysosporium* was capable of degrading phenolic compound present in the pod coffee. 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 pod coffee after cultivation.

The tannin degrading ability of *P. chrysosporium* on pod coffee (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 fermentation mentation, and the physiological behaviour difference (Akinfemi and Ogunwale, 2012).

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

Table 2. Changes of nutrien contents of pod coffee substrate fermented by *Phanerocaete chrysosporium* before and after fermentation (as % dry matter)

Nutrient	Before fermentation	After fermentation	%
Organic matter	92.71	85.6	-7.59
Crude protein	10.36	12.64	17.2
Neutral detergent fiber	94.18	78.08	-16.6
Acid detergent fiber	86.18	74.08	-15
Hemicellulosa	7.99	5.32	-33.41
Cellulose	18.51	23.8	27.11
Lignin	64.42	44.04	-31.12
Tannin	1.02	0.18	-82.35
Caffeine	1.39	0.2	-85.61

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

Parameter	R0	R1	R2	R3	R4
pH^{ns}	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)^{ns}	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}

Means in the same row with different superscript differ significantly (P<0.05). Level of fermented pod coffee : R0 (0%/control), R1 (1%), R2 (2%), R3 (3%), R4 (4%).

***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 of pod coffee. 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 of pod coffee 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 decrease of lignin levels was due to the activity of the LiP and MnP enzymes produced by the *P. chrysosporium*, they were able to degrade the fiber fraction so that the previously strong bonds became tenuous. The decrease in lignin content after fermentation is expected to be large because it means that the level of degraded lignin is large. The greater the degradation of lignin in the feed, the better in increasing feed digestibility.

The structural carbohydrate (cellulose and hemicellulose) in fermented pod coffee 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 pod coffee level may indicate that inclusion fermented of pod coffee to 40% in diets does not interfere with ruminal protein metabolism.

The ruminal protozoa population in control diets was higher than the fermented pod coffee diets (P<0.05). No statistically difference were observed in ruminal protozoa among fermented pod coffee 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 (Iwalokun *et al*., 2007; Chan *et al*., 2009).

The inclusion of fermented of pod coffee in the diets decreased the dry matter digestibility (P<0.05). This is due to the activity of microbes in the fermentation activity in the coffee pod can produce CO_2 resulting in decreasing water content. The inclusion more than 20% fermented of pod coffee 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 and 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). Tannins are polyphenolic compounds that can protect proteins from rumen microbial degradation by protease enzymes. Tannins are also a limiting factor for protein absorption because of their ability to bind tannins to form protein-tannin bonds.

Conclusions

The treatment of pod coffee with *P. chrysosporium* 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 of pod coffee diets decrease the ruminal protozoa. Volatile fatty acid and dry matter digestibility decreased as the level of fermented pod coffee increased.

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