ISSN: 0852-3681
E-ISSN: 2443-0765

Jurnal Ilmu-Ilmu Peternakan 32(3): 416 – 426
Available online at http://jiip.ub.ac.id

Evaluation of coffee bean husk fermented by a combination of *Aspergillus niger, Trichoderma harzianum*, and *Saccharomyces cerevisiae* as animal feed

Himmatul Khasanah*[1)], Desy Cahya Widianingrum[1)], Listya Purnamasari[13)] and Ali Wafa[2)], Seong-Gu Hwang[3)]

[1)]Animal Husbandry Program Study, Faculty of Agriculture, University of Jember, Jember 68121 Indonesia
[2)]Plant Protection Program Study, Faculty of Agriculture, University of Jember, Jember 68121 Indonesia
[3)]School of Animal Life Convergence Science, Hankyong National University, Hankyong National University, Anseong-si, Gyeonggi-do 17579, Republic of Korea

Submitted: 05 April 2022, Accepted: 19 December 2022

ABSTRACT: Abundant coffee bean husk acquires an alternative source of fiber for livestock feed, but a high level of the crude fiber of it became an obstacle. Solid-state fermentation technology using lignocellulolytic fungi is known to be able to improve the nutritional quality of feedstuff that have high fiber content. Its mechanism is through the degradation of the lignocellulose fraction and enhance protein content. This study aimed to determine the nutritional quality of fermented coffee bean husk with a combination of fungi and yeast. The fermentation method used a solid-state fermentation consisting of 7 different inoculums, namely: P0: Unfermented coffee bean husk, P1: *Aspergillus niger*, P2: *Saccharomyces cerevisiae*, P3: *Trichoderma harzianum*, P4: *Aspergillus niger + S. Cereviciase*, *P5: Aspergillus niger + Trichoderma harzianum*, P6: *Saccharomyces cerevisiae + Trichoderma harzianum* and P7*: Aspergillus niger + Saccharomyces. Cereviciase + Trichoderma harzianum*. The nutritional quality of the fermented coffee bean husk was determined by proximate analysis, lignocellulolytic fraction, and digestibility. The data obtained were analyzed by ANOVA and followed by Tukey's post hoc test. The crude fiber content of fermented coffee bean husk (P1-P7) was lower than unfermented (P0). There was no significant difference among treatments in crude fat and protein. Treatment P3 has the highest total digestibility nutrient (70) and the lower crude fiber (15.03). A combination of *Aspergillus niger* and *Saccharomyces cerevisiae* reduce lignin content by about (4,16%). In conclusion, the fermented coffee bean husk can be utilized as animal feedstuff with higher nutritional quality than unfermented.

Keywords: Local feedstuff; Lignocellulolytic fungi; Solid state fermentation; Yeast

*Corresponding Author: himma@unej.ac.id

INTRODUCTION

Recently, increased product demand and consumption have emerged in the coffee industry. Along with the growth of this industry, excess organic waste is produced from wet and dry processing activities. Wet processing generates coffee pulp and parchment husk, while dry processing creates waste as coffee husk (Oliveira and Franca, 2015). The coffee bean husk is rich in nutrients based on its proximate analysis, specifically the fiber content that has the potential as an alternative animal feed (Iriondo-DeHo et al. 2020). Furthermore, coffee bean husk contains carbohydrates, i.e. sugar, polyphenols, and fats that can be used as carbon sources during the fermentation process by microorganisms (Kumar et al. 2018; Khasanah et al. 2020).

Many microbes can be used as starters for feed fermentation, usually bacteria, fungi, or yeast which can be beneficial and have cellulolytic properties. *Trichoderma harzianum* has a potential as the SSF (Solid-State Fermentation) starter. This cellulolytic fungus can deteriorate cellulose efficiently and is frequently used to produce enzymes (Benoliel et al. 2013; Das and Abdulhameed 2020). The fermentation process using *Trichoderma harzianum* has been performed on several agricultural wastes, namely banana, orange peel, tomato, apple (Siada et al. 2018), sunflower lignocellulosic fraction (Parrado and Bautista, 1993), cassava-based feed (Vuong et al. 2021), and rice polishing (Ahmed et al. 2017). This fermentation process is proposed to increase the protein content for further utilization as animal feed and various feed ingredients. Meanwhile, *Aspergillus niger* is also used as an SSF starter on *Ginkgo biloba* leaves to compose natural feed additives (Wang et al. 2018). Utilization *of Aspergillus niger* in rapeseed cake fermentation decreased the neutral detergent fiber (NDF), glycosylate, and phytic acid up to 9.12, 76.89, and 44.60%, respectively. In contrast, this fermentation process increased the crude protein, soluble protein, and ether extract contents of the cake up to 23.02, 23.71, and 23.54% (Shi et al. 2015). The combination of *Aspergillus niger* and *Rhizopus oligosporus* improved the proximate value of fermented cassava and tofu mixture for broiler chicken feed ingredients (Yohanista et al. 2014). The use of fermented bran with *Aspergillus niger* in broiler chicken feed ingredients promoted better intestinal properties by reducing the pH level, reducing intestine viscosity, and boosting the proteolytic enzyme without any significant influence on the organ digestion weights (Supartini and Fitasari, 2011). *Aspergillus niger* is classified as a non-pathogenic fungus and reported safe for livestock digestive tract when the spores are inhaled (Schuster et al. 2002).

Another microbe that is usually used as a starter and have probiotic ability is yeast. The most famous one is *Saccharomyces cerevisiae* which has favorable properties for fermentation. *Saccharomyces cerevisiae* fermented product is widely used as a ruminal feed. This product could improve the nutrient level, energy, and milk production of a dairy cow by promoting proteolytic, cellulolytic, and lactate-utilizing bacteria in the rumen (Callaway and Martin, 1997). Supplementation of fermented feed with *Saccharomyces* could increase the daily feed intake, milk fat content, modified cholesterol metabolism, and prolonged subclinical ketosis occurrence. However early-lactation milk yield and metabolism remained unaffected (Olagaray et al. 2019). According to Nuryana et al. (2016), the fermentation process of coffee bean husks using a combination of *Rhizopus oryzae* and *Saccharomyces cerevisiae* could increase crude protein and crude fiber contents by 16.99 and 16.28%, respectively.

In this study, we aimed to analyze the effect of SSF on the nutritional quality of coffee bean husk. By using the SSF method and combined inoculum, we expect that this method can enhance the proximate and digestive value of the coffee bean husk, followed by discovering the most desirable inoculum mixture between single or combination cellulolytic fungi. Accordingly, this study describes the optimized utilization of agricultural waste, particularly in the coffee

plantations area integrated with coffee plantations and livestock. Furthermore, this study also promotes the zero-waste program of the integration process.

MATERIALS AND METHODS

Materials

The sample was Robusta coffee bean husk from PTPN XII Plantation, Rembangan, Jember Regency, Indonesia. The culture used were *Aspergillus niger (*FNU 6018), *Saccharomyces cerevisiae* (FNCC 3012) obtained from The Research Center for Biotechnology, Universitas Gadjah Mada, and *Trichoderma harzianum* obtained from Plant Protection Laboratory, Faculty of Agriculture, University of Jember. The dried cultures were refreshed in Potato Dextrose Agar (PDA), Merck Sigma-Aldrich, Germany. Each isolate was then cultured again in Potato Dextrose Agar, Merck Sigma-Aldrich, Germany, and incubated at 35℃ temperature for two days.

Coffee Bean Husk Fermentation with Solid-State Fermentation Method

Coffee bean husk samples were sun-dried and then sterilized using an autoclave at 121°C for 15 minutes. The starter mixture was composed of the isolate dissolved in 285 ml water and 15 ml molasses. This solution was stirred until homogeneous, then added to the substrate (sterilized coffee bean husk). Each treatment contained 500 g sterile substrate moisturized with 60% water (60% x 500 g = 300 ml water) and mixed with the starter according to the treatment (Table 1) and packed in the plastic with holes on each end and middle of the plastic, before incubating at room temperature for 20 days (Daning et al. 2018). All treatments were replicated four times.

Table 1. The treatment of solid-state fermentation

Treatment	Starter
P1	*Aspergillus niger*
P2	*Saccharomyces cerevisiae*
P3	*Trichoderma harzianum*
P4	*Aspergillus niger + Saccharomyces Cereviciase*,
P5	*Aspergillus niger + Trichoderma harzianum*
P6	*Saccharomyces cerevisiae + Trichoderma harzianum*
P7	*Aspergillus niger + S. Cereviciase + Trichoderma harzianum*

Fermented coffee bean husk nutrient quality analysis

The whole fermentation products which were mostly in solid form were analyzed for nutrient content using the proximate analysis method, containing moisture, ash, crude fiber, and crude fat. Meanwhile, crude protein content was analyzed based on the Kjeldahl method (AOAC, 1990). The lignocellulose level was determined based on the Chesson method (Datta, 1981). The dry and organic matter digestibility were evaluated according to Tiley and Terry (1963). The scanning electron microscope (SEM) analysis was conducted to visualize fungal presence using *TM3030 Plus 005* Tabletop Microscope (Benchtop SEM).

Data analysis

The data were analyzed by adopting a Completely Randomized Design (CRD) with seven treatments (Table 1). If there was a significant effect among the treatments, a Post Hoc Tukey test was applied, and statistical analysis was performed using SPSS IBM version 26.0 SPSS Inc., Chicago, USA.

RESULT AND DISCUSSION

The SSF among various treatments determines the nutritional quality of coffee bean husk (Table 2). Our study revealed that all treatments decreased ash and crude fiber content. Furthermore, crude fat and protein contents obtained an insignificant difference among treatments. In contrast, the nitrogen-free extract and total digestible nutrient

(TDN) increased among treatments both on a single and combined starter.

The lignocellulolytic fraction also varied among treatments. Hemicellulose levels in P4 and P6 treatments increased, following a decreased lignin level in both treatments. Moreover, the cellulose level declined in the P1, P2, P3, and P4 treatments. Table 3 describes that the most effective treatment to decrease lignin levels is the P4 treatment.

Table 2. Nutritional characteristics of fermented coffee bean husk

Treatment	Moisture (%)	Ash (%)	Crude Fiber (%)	Crude fat (%)	Carbohydrate (%)	Crude protein (%)	Nitrogen-free extract (%)	TDN
P0	18.56 ^{ab}	10.50^{c}	18.76 ^{c}	1.55 ^{ns}	20.35^{ab}	10.35 ^{ns}	59.40 ^{a}	66.29^{a}
P1	21.25^{de}	7.60^{a}	15.65^{ab}	1.47^{ns}	17.76^{a}	12.67 ^{ns}	62.86^{ab}	69.52^{bc}
P2	20.22^{cd}	7.54^{a}	15.03^{ab}	1.62 ^{ns}	18.05^{a}	9.71 ^{ns}	65.82^{b}	70.00^{bc}
P3	22.41^{e}	7.27^{a}	13.93^{a}	1.60 ^{ns}	19.26^{ab}	10.58 ^{ns}	66.32^{b}	71.07^{c}
P4	17.93^{a}	7.49^{a}	15.74^{b}	1.67 ^{ns}	18.42^{a}	10.11 ^{ns}	64.89^{b}	69.30^{b}
P5	21.41^{cd}	7.26^{a}	14.49^{ab}	1.72 ^{ns}	21.35^{b}	10.68 ^{ns}	65.99^{b}	70.57^{bc}
P6	19.84^{bc}	8.39^{b}	14.80^{ab}	1.62 ^{ns}	20.43^{ab}	10.26 ^{ns}	64.63^{ab}	69.93^{bc}
P7	19.24^{cd}	9.90^{c}	15.00^{ab}	1.42 ^{ns}	20.49^{ab}	12.06 ^{ns}	62.11^{a}	69.75^{bc}

P0= Unfermented, P1 = *Aspergillus niger*, P2= *S. cerevisiae*, P3= *Trichoderma harzianum*, P4= *Aspergillus niger + S. cerevisiae*, P5= *Aspergillus niger+ Trichoderma harzianum*, P6= *S. cerevisiae+ Trichoderma harzianum*, P7= *Aspergillus niger+ S. cerevisiae+ Trichoderma harzianum.* Different superscript letters show a significant different at 5% confidence level (P <0.05).

Table 3. Lignocellulolytic fraction of fermented coffee bean husk

Treatments	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Unfermented (P0)	7.48^{ab}	35.20^{e}	40.27^{d}
Aspergillus niger (P1)	7.75^{abc}	30.79^{ab}	39.40^{cd}
Saccharomyces cerevisiae (P2)	8.52^{bcd}	32.32^{bcd}	39.36^{cd}
Trichoderma harzianum (P3)	7.87^{bc}	29.97^{a}	40.29^{d}
Aspergillus niger+ Saccharomyces cerevisiae (P4)	8.86^{cd}	32.49^{cd}	36.11^{a}
Aspergillus niger+ Trichoderma harzianum (P5)	7.58^{ab}	33.89^{de}	38.67^{bcd}
S. cerevisiae+ Trichoderma harzianum (P6)	9.28^{d}	33.51^{de}	37.28^{ab}
Aspergillus niger+ S. cerevisiae+T. harzianum (P7)	6.69^{a}	31.44^{abc}	38.60^{bc}

Note = Different superscript letters show a significant difference at a 5% confidence level (P <0.05).

The feedstuff quality can be evaluated by observing the dry matter and organic matter digestibility values. These digestibility values demonstrate the number of nutrients that the digestive tract can absorb. Our result showed that dry matter and organic matter digestibility values of fermented coffee bean husk were low (below 40%). Descriptively, the highest digestibility value was obtained from a single inoculum of *Trichoderma harzianum* (P3).

Optimum fiber-bond breakdown and lignocellulosic hydrolysis of feed ingredients indicate a good fermentation process, which can improve the digestibility value. Based on this study, there was a change in the surface structural alteration that occurred on the fermented coffee bean husk (Figure 1).

The P0 treatment shows ~~a~~ rough and textured surface. Fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae* (Figure 1, P4) shows a textured coffee bean husk surface filled with hyphae. *Saccharomyces cerevisiae* and *Trichoderma harzianum* combined fermentation show an antagonistic condition as same as *Aspergillus niger* and *Trichoderma harzianum* combined fermentation as the SEM image shows damaged *Aspergillus niger* hyphae and less colony of *S. Cerevisiae* (Figure 1 P7).

In this study, *Aspergillus niger*, *Trichoderma harzianum*, and *Saccharomyces cerevisiae* are known as cellulolytic microorganisms (Godoy et al. 2018, Sekah et al. 2018). Table 2 shows the proximate analysis results of fermented coffee bean husk on different cultures of inoculum, which produce different nutrient qualities. The moisture content of unfermented coffee bean husk was 18.56%, which increased after the fermentation process as observed in all treatments, except in the P4 and P6 treatments.

Table 4. In vitro digestibility value of fermented coffee bean husk

Treatments	Dry matter digestibility (%)	Organic matter digestibility (%)
Aspergillus niger (P1)	36.26	34.10
Saccharomyces cerevisiae (P2)	38.15	35.56
Trichoderma harzianum (P3)	39.86	36.91
Aspergillus niger+ *Saccharomyces cerevisiae* (P4)	36.14	34.27
Aspergillus niger+ *Trichoderma harzianum* (P5)	36.68	34.85
S. cerevisiae+ *Trichoderma harzianum* (P6)	37.25	25.29
Aspergillus niger+ *S. cerevisiae*+*T. harzianum* (P7)	35.83	33.63
48-hour-fermented rice straw with 5% *Aspergillus* [1]	25.95	36.99
72-hour-fermented rice straw with 5% *Aspergillus*[1]	35.04	42.13
Ammoniated corn husk + 5% *Aspergillus niger* after 2 weeks of fermentation[2]	66.50	67.64
75% Corn straw +25% rice bran + 1% *Aspergillus niger*	77.46	70.80

Note = Data in the table were not statistically analyzed; [1]Saputro et al. (2015); [2]Septianto et al. (2019)

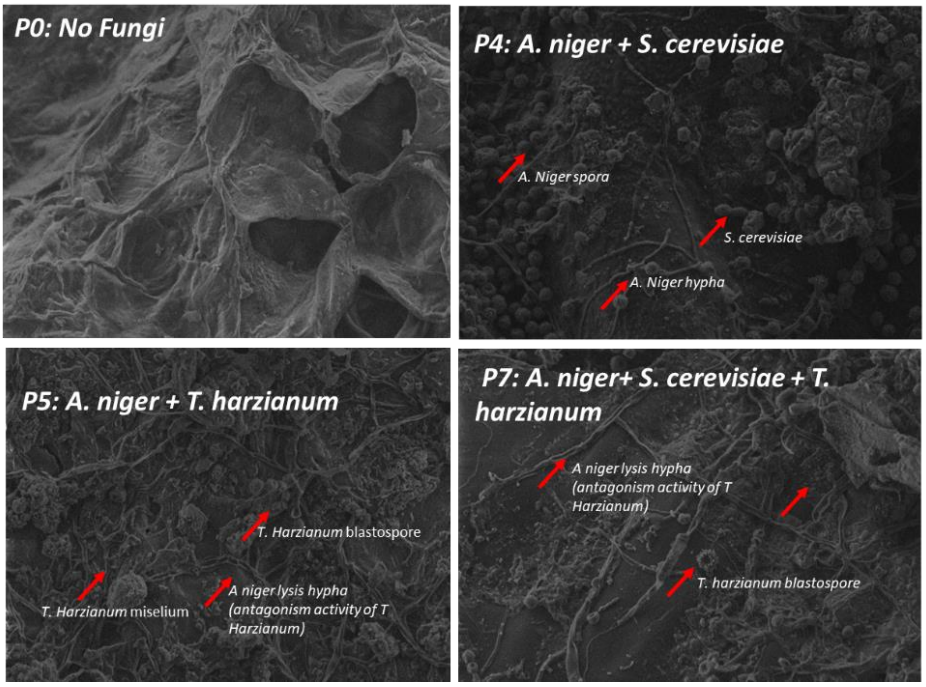

Figure 1. The compatibility condition of *Aspergillus niger* and *Saccharomyces cerevisiae* (P4). Incompatibility condition between *Aspergillus niger* and *Trichoderma harzianum* (P7)

Increased moisture content was caused by the water addition before the fermentation process and from the fungi metabolism (Gervais and Molin 2003). The crude fat content of fermented and unfermented coffee bean husk obtained an insignificant difference. This condition followed the result of a coffee husk fermented with *Saccharomyces cerevisiae*, which did not show an increased crude fat content (Dinata and Utami 2019).

Carbohydrate content in fermented coffee bean husk showed a significant difference as the P5 obtained a carbohydrate content of 21.35%, which was higher than the P1, P2, and P4. Several treatments obtained a reduced carbohydrate due to the use of carbon by microorganisms for metabolism, including lignocellulolytic hydrolysis using cellulase to become a simple molecule (glucose). This fermentation process proves the reduction of crude fiber content (Dinata and Utami, 2019). The cellulose in the substance was used as an energy source for microbes. This result is similar to Oshoma and Eguakun-Owie (2018), who reported that fungal metabolism through the use of carbohydrates for growth required a high amount of carbohydrates to accelerate fungal growth. Cellulose hydrolysis into glucose is influenced by the incubation period. MacLellan et al. (2010) described that during the incubation period, inoculum released inhibitory compounds irreversibly to lignin enzyme absorption as enzymatic cellulose conversion inhibitors to sugar monomers.

The crude protein contents of fermented coffee bean husk were insignificantly different among treatments. This may occur as a result of the inoculum addition with a similar concentration at 12%. Nitrogen/urea supplementation can be used as a source of nutrients in culture to assign the fermentation process (Junges et al. 2017). The fermentation period also influences fungal growth, which can be possibly utilized as a Single Cell Protein (SCP). The fermentation effective period is varied among inoculants as a longer fermentation period will decrease the crude protein content in a fermented substrate (Mihrete and Bultosa 2017). However, Oshoma and Eguakun-Owie (2018) reported that SCP production using *Aspergillus niger* required a shorter fermentation period, namely 5 days. Decreased crude protein during fermentation can be caused by the conversion of free amino acids to amines used for fungal protein synthesis (Dinata and Utami 2019). Moreover, Zhu et al. (2017) reported that the supplementation of low-quality forage feed fermented using *Saccharomyces cerevisiae* could increase N conversion without changing rumen pH and N-ammonia content, in addition to enhancing fatty acids, acetate, propionate, and butyrate contents. Feed processing through SSF using *Aspergillus niger* inoculum could increase the total amino acids and essential amino acids in rapeseed and improve endoglucanase, xylanase, acidic protease, and phytase enzyme activities to reduce neutral detergent fiber (NDF) levels (Shi et al. 2015).

The fermentation process also influenced the cellulose fraction. This study showed that the hemicellulose content in the P7 was lower than the P3, P2, P4, and P6. The interaction between fungi and yeast in the P7 obtained the most effective results to degrade hemicellulose fraction. In contrast to the cellulose fraction, the P1, P2, P3, P4, and P7 obtained a better result than the P0 (without fermentation) and P3 treatments, which obtained the lowest degradation value but were statistically similar to the P1 treatment. For lignin fraction degradation, the most effective treatment was obtained from the P4. Based on the lignocellulose content analysis, lignin degradation was inversely proportional to cellulose degradation (Figure 1). *Aspergillus niger* has the best capability to degrade lignin to cellulose and hemicellulose (Khan and Dwivedi 2013). Cellulose is the polymer of β-1,4- linked glucose monomers which can be hydrolyzed into glucose. Furthermore, hemicellulose is a heteropolysaccharide composed of xylose, mannose, and glucose with sidechains of arabinose, acetyl groups, or glucuronic acid. Between cellulose and hemicellulose, there is lignin which is an aromatic residual ma-

trix (Ralph et. al. 2019). These three polysaccharides are the main formation materials of the cell wall that are difficult to digest. The hydrolysis of cellulose and hemicellulose requires a synergistic action between the extracellular enzymes (Do Vale et al. 2014).

About 35% of plant cell walls are composed of hemicellulose which can be used as feedstuff. In addition, the crude fiber fraction that is widely used by ruminants is cellulose. *Trichoderma* and *Aspergillus* arebroad-spectrum fungi that can produce cellulose-degrading enzymes (Beckham et al. 2012). Most of these enzymes are free enzymes that bind to the substrate using carbohydrate-binding modules. However, several microorganisms are known to have cellulosomes as a set of cellulose-degrading enzymes produced by anaerobic fungi including *Trichoderma* (Do Vale et al. 2012; Silva, 2012). Moreover, several fungi exist in the ruminant digestive tract (Bayer et al. 2004; Do Vale et al. 2014).

Trichoderma is known to be able to secrete cellulolytic and xylanolytic enzymes, such as cellobiohydrolase, arabinofuranosidase, chitinase, endo-xylanase, endo-glucanase, and swollenin (Do Vale et al. 2014). The production of cellulase enzymes by *Aspergillus niger* in sugarcane and pollard bagasse substrate produced immense cellulase (avicelase and CMCase) and FPase activities on the third and fourth day of fermentation, followed by the maximum production of β-glucosidase and xylanase on the fifth day (de Oliveira Rodrigues et al. 2020).

In connection with the production of VFA (Volatile Fatty Acid) during feed ingredient fermentation with rumen, the fermentation process of feed ingredients with white rot fungi provides various effects, as high lignin content in feed ingredients declines the VFA production, but high cellulose content in feed ingredients inclines the VFA content (Fang et al. 2020). *Trichoderma harzianum* had a prominent inhibitory impact on the production of another fungus in dual culture (P5 and P7, Fig 1). According to Lone et al. (2012) *Trichoderma harzianum* caused the maximum growth repression in *Aspergillus niger* approximately 75%.

The digestibility level of fermented coffee bean husk and several local feeds from agricultural waste are shown in Table 4. This result showed that the digestibility of fermented coffee bean husk was lower than other feedstuffs. Based on our findings, the utilization of fermented coffee bean husk must be formulated with other local feedstuff to the obtained nutrient requirement of animals.

Heterogenous digestibility values of feedstuff require more effort to formulate a balanced ration containing supplements and feed ingredient mixture to fulfill the nutrient requirements of the livestock (Mayulu, 2012). Feed ingredient digestibility is influenced by several factors, particularly the feed ingredient form, composition, and treatment (Priyanto et al. 2017). The higher the inoculum's capability to degrade fiber bonds, the higher the digestibility level of the fermented product. Based on the nutrient properties, the fermented coffee bean husk can be used as a source of fiber and protein feed for ruminants.

CONCLUSIONS

It can be concluded that, in general, fermentation with a combination of fungi and yeast showed various results, and it can reduce the crude fiber contents of coffee bean husk. The crude fat and protein content were not significantly different in all treatments. Fermentation with *Trichoderma harzianum* has the best method to improve nutritional quality in terms of reducing crude fiber and increasing the total digestibility of nutrients. Intensively, using a combination inoculum of *Aspergillus niger* and *Saccharomyces cerevisiae* for fermentation to reduce lignin content.

Fermentation results also show an inverse trend between lignin and cellulose degradation. The usage of fermented coffee bean husk needs a combination with other local feedstuffs.

ACKNOWLEDGMENTS

This work was supported by the Research Supporting Grant Program, at the University of Jember with grant number 6707/UN25.3.1/LT/2019.

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