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(Isolated from of Bali Cattle Colon) as Feeding of Broiler**

By

**Gusti Agung Oka Citrawati, I.G.N.G. Bidura and
Dewi Ayu Warmadewi**

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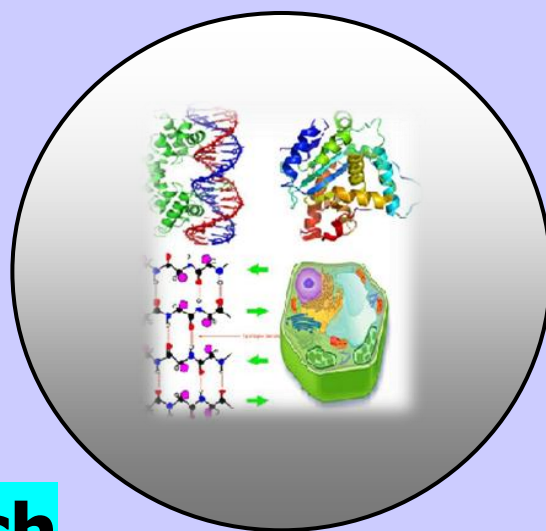
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RESEARCH PAPER

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The Increase of Nutrient Quality of Coffee Pulp by Fermenting with *Saccharomyces Sp.Sb-6* (Isolated from of Bali Cattle Colon) as Feeding of Broiler

Gusti Agung Oka Citrawati, I.G.N.G. Bidura and

Dewi Ayu Warmadewi

Magister Programs of Animal Science, Faculty of Animal Science, Udayana University,
Denpasar, Bali-Indonesia

Jln. PB. Sudirman, Denpasar, Bali, Indonesia

ABSTRACT

This research was carried out to study the increase of nutrient quality of coffee pulp by fermenting with Saccharomyces sp.Sb-6 (isolated from of bali cattle colon) as feeding of broiler. The research used a complete randomized design (CRD) with three treatments in six replicates. The third treatment were coffee pulp unfermented as control (A); coffee pulp with 0.20% Saccharomyces sp.Sb-6 fermented (B); and coffee pulp with 0.40% Saccharomyces sp.Sb-6 fermented (C), respectively. The results of this experiment showed that coffee pulp fermentation with 0.20-0.40% Saccharomyces sp.Sb-6 were significantly different ($P<0,05$) increased crude protein, crude fibre, and gross energy of coffee pulp. The coffee pulp fermented by 0.20-0.40% Saccharomyces sp.Sb-6 were significantly different ($P<0,05$) improved dry matter digestibility, organic digestibility, and protein digestibility rather than control. It can be concluded that fermentation of coffee pulp by Saccharomyces sp.Sb-6 (isolated from bali cattle colon) can improve of nutrient and digestibility of coffee pulp as broiler feeding

Key words: Fermentation, *Saccharomyces Sp.Sb-6*, Digestibility and Coffee.

INTRODUCTION

Indonesia is the third coffee producer in the world, behind Brazil and Vietnam. Evidently, in 2012, Indonesia is able to produce at least 748 thousand tons or 6.6% of the world coffee products. Coffee is produced from coffee plantation which covers 1.3 million hectares in Indonesia, including coffee plantations in Bali (Hartono, 2013). Potential land for coffee development in Bali is still quite large, especially in the central area of the plantation and

marginal land. In the province of Bali (Indonesia) are plantations covering an area of 123771 ha, and 29% is a coffee plantation.

Based on the large number of existing coffee, of course, coffee processing will produce a lot of waste. By-products of the coffee fruit, including fruit pulp (48%), which consists of 42% of the skin of the fruit and 6% is the seed coat. It was also reported that the coffee skin contains 10.4% crude protein, crude fiber 17.2%, and metabolizable energy 14.43 MJ / kg. Meanwhile, according to Simanihuruk *et al.* (2010), the proportion of skins produced in the processing of coffee is quite large, ie 40-45% of the total coffee fruit.

According Budiari (2014), coffee pulp waste utilization as feed needs to be studied because it contains sufficient nutrients for the animals. High crude fiber content of the skin coffee limiting factor in its use as poultry feed. Therefore it is necessary for the processing of fermentation. According Bidura (2007), the advantage of fermentation is changing macro protein molecules into micro molecules that are easily digested by birds, and does not produce toxic chemical compounds. It was also reported, in addition to improving the protein content in rations, the fermentation process can also increase digestibility of feed and can be untied complex compounds into compounds that are easily digested.

Fermentation of fiber in feedstuffs will be able to increase the nutrient content of the feed. Fermentation can improve the digestibility of dry matter, organic matter, crude protein and crude fiber materials. Wibawa *et al.* (2015) reported that the fermentation of rice bran with 0.20 to 0.40% yeast *Saccharomyces* sp complex can increase the nutrient content and digestibility of rice bran as feed ducks. Unfortunately this by-product contains toxic factors such as phytic acid as phytate and high content of crude fiber. These anti-nutritive factors have been reported by Kahlique *et al.* (2003) causing reduction of feed intake and depress performance of poultry. These anti-nutritive factors have been reported to reduce feed intake and depress performance of poultry. Bach Knudsen (2001) reported that CF has been defined as the complex macromolecular substances in food plants that are not degraded by poultry digestive enzymes. With the exception of lignin, all of the materials called CF are carbohydrates in nature. Crude fibre is thought to mediate protective effects on the colonic epithelium through their fermentation products and fecal bulking capacity (Wang *et al.*, 2004). Feeding high fiber resulted in a lowered rate of lipogenesis and tendency of an increased capacity to utilize acetyl-CoA in pigs (Zhu *et al.*, 2003). Non starch polysaccharides (NSP) are the carbohydrate components of CF and are the predominant substrates for anaerobic fermentation.

The potency of coffee pulp (48%), as energy source for poultry depends on its cell wall content, degree of microbial fermentation in poultry large intestine, absorption and production of the volatile fatty acid (Wang *et al.*, 2004). The potensial of coffee pulp as energy sources for poultry depends considerably on such factors as cell wall content, degree of microbial fermentation in the large intestine, and extent of absorption and utilization of the volatile acids produced (Kahlique *et al.*, 2003). The use of coffee pulp in poultry diets has been highly limited by the presence of phytic acid and other minor anti-nutritional factors like tannin and non-starch polysaccharides (NSP). The high levels of NSP in coffee pulp are limiting its unrestricted use in poultry feeding. These NSPs are known to increase the gut viscosity, reduce nutrient absorption in the intestine and affected indirectly the growth and performance of birds (Rhames *et al.* 2006; Cao *et al.*, 2003).

Many studies have clearly demonstrated that, supplemented of probiotics to diets rich in NSP results in a significant reduction in the intestinal viscosity, enhances energy, and protein utilization (Bidura *et al.*, 2012; Bidura *et al.*, 2009). Hong *et al.* (2004) reported that fermentation of feed using *Aspergillus oryzae* increased digestibility of its DM and CP.

The use of *Saccharomyces cerivisiae* (Sc.) culture as a probiotics sources in poultry production as become an area of great interest, because continued use of probiotics in animal feeds may result in the presence of antibiotics residues in animal products (Han *et al.*, 2001). Gut microfloral enzymes are beneficial to the nutrition of the host because they increase the digestion of nutrients, especially in the lower intestine. Previous experiments showed that the inclusion of microorganisms in the diets improved feed conversion efficiency and digestibility (Chen *et al.*, 2005). Piao *et al.* (1999) showed that 0.10% yeast added to a diet could reduce animal wastes. Yeast culture improved phosphorus utilization in growing chickens.

This research was carried out to study the increase of nutrient quality of coffee pulp by fermenting with *Saccharomyces sp.Sb-6* (isolated from of bali cattle colon) as feeding of broiler.

MATERIAL AND METHODS

Animals and experimental design

Eighteen of broilers up to six weeks of age were assigned to three treatments in a completely randomized design. Each treatment has six replications with one bird per replication (individual cage). All of the birds were fed experimental diets for two days. The treatments were (i) coffee pulp unfermented as control; (ii) coffee pulp fermented by *Saccharomyces spp.Sb-6 isolate*; and (iii) coffee pulp fermented by *Saccharomyces spp.Sb-6 isolate*, respectively. The objectives of this study is to determine the nutrient digestibility and the metabolizable energy value of coffee pulp using broiler up to six weeks of age.

Fermented of Coffee Pulp

The isolate of *Saccharomyces spp.Sb-6* which has been passed approved from bile salt test and *in vitro* test on poultry digestive tract, could assimilate cholesterol, and both as probiotics agent and has CMC-ase activity too. The isolate has passed the test and has been considered as potential probiotic according to our previous study (Bidura *et al.*, 2014). Fermentation of coffee pulp (by-product) was prepared as follows: coffee pulp was added approximately 0.40% (2×10^7 spores) *Saccharomyces spp.Sb-6* into the 100 g of steamed coffee pulp. Then, water was added until the content of water up to 35% and for 2 days for incubations. After that, coffee pulp fermented was dried at 45°C temperature for six hours and then were prepared for analysis.

Retention and excretion of nutrients

In order to determine the nutrient digestibility and metabolizable energy (ME) of the coffee pulp (by-product). The amount of coffee pulp used was 50 g. This amount as based on preliminary assays with broilers aged to six week using coffee pulp. All of the birds were deprived of feed for 24 h to ensure that their alimentary canals were empty from feed residues. They were then forced-fed with the specific amount of coffee pulp (fermented and unfermented).

Stainless steel funnel with 40 cm stem was used in forced feeding technique (Mustafa *et al.*, 2004). Water was offered *ad libitum* during the experimental period. The total excreta were collected in plastic trays. The excreta samples were frozen, to equilibrium with the atmospheric moisture, weighed, and grinded through a 1 mm sieve. Samples of excreta and coffee pulp were subjected to appropriate analysis to determine DM, OM, CP, CF, and energy, respectively.

Laboratory analyses

To determination of Dry matter (DM), organic matter (OM), CP and ash were done according to the Association of Official Analytical Chemists (1994). The content of CP samples was determined using the Kjeldahl procedure (AOAC, 1994). Crude fibres in the samples were determined using the procedure of Van Soest *et al.* (1991) on oven-dried samples. Gross energy (GE) was measured with an adiabatic oxygen bomb calorimeter (Parr, USA).

Calculations

The data were used to calculate AME value according to the following formulate (Mustafa *et al.*, 2004): AME (apparent metabolizable energy) = IE – FE. Where IE = ingested energy; FE = fecal energy voided by the fed birds.

Statistical Analysis

All data were subjected to analysis of variance and if it was significantly different it was further tested by Duncan's Multiple Range Test.

RESULTS

The results showed that the dry matter content of coffee pulp without fermentation (A) is 88.43%. The average dry matter content of coffee pulp fermentation with *Saccharomyces sp.Sb-6* 0.20% (B), and coffee pulp fermented by 0.40% *Saccharomyces sp.Sb-6* (C were 0.06% and 0.23% had no significant ($P>0.05$) was higher than the nutritional value of coffee pulp without fermentation (A), respectively. Chemical composition of coffee pulp (fermented compared to the unfermented) were shown in Table 1 as below:

Table 1. Effect of coffee pulp fermentation with *Saccharomyces sp.Sb-6* on the content of dry matter, organic matter, crude protein, crude fiber, and gross energy of coffee pulp.

Variable	Treatment ¹⁾			SEM ²⁾
	A	B	C	
Dry matter (%)	88.43 ^{a3)}	88.48 ^a	88.64 ^a	0.07
Organic matter (%)	92.30 ^a	92.82 ^a	93.13 ^a	0.21
Crude protein (%)	11.72 ^b	13.01 ^a	13.08 ^a	0.12
Crude fibre (%)	23.90 ^a	22.42 ^b	22.14 ^b	0.17
Gross energy (GE kcal/kg	3156.17 ^b	3502.67 ^a	3392.50 ^a	41.87

Note:

- 1) Coffee pulp without fermented as a control (A), coffee pulp fermented by 0.20% *Saccharomyces sp.Sb-6* (B), and fermented by 0.40% *Saccharomyces sp.Sb-6* (C)
- 2) SEM: Standart Error of The Treatment Means
- 3) The different superscript at the same row is significantly different ($P<0.05$)

The results showed that the crude protein value of coffee pulp without fermentation (A) was 11.72% (Table 1). The mean value of crude protein coffee pulp fermentation with 0.20% *Saccharomyces sp.Sb-6* (B), and coffee pulp by 0.40% fermented *Saccharomyces sp.Sb-6* (C) were 11.05% and 11.65% ($P<0.05$) significantly different higher than the nutritional value of coffee pulp without fermentation (A), respectively.

The content of crude fiber coffee pulp without fermentation (A) was 23.90% (Table 1). The mean value of crude fiber coffee pulp with 0.20% *Saccharomyces sp.Sb-6* (B), and coffee pulp by 0.40% fermented *Saccharomyces sp.Sb-6* (C) were 6.21% and 7.40% ($P<0.05$) significantly different lower than the nutritional value of coffee pulp without fermentation (A), respectively.

The content of gross energy coffee pulp without fermentation (A) was 3156.17 kcal/kg (Table 1). The average gross energy value of coffee pulp fermentation with 0.20% *Saccharomyces sp.Sb-6* (B), and coffee pulp fermented by 0.40% *Saccharomyces sp.Sb-6* (C) were 10.98% and 7.49% different ($P<0.05$) significantly higher than control (A), respectively. The results showed that dry matter digestibility value of coffee pulp without fermentation (A) was 25.64% (Table 2). The average value of dry matter digestibility coffee pulp fermentation with 0.20% *Saccharomyces sp.Sb-6* isolates from bali cattle colon (B), and coffee skin fermented by 0.40% *Saccharomyces sp.Sb-6* isolates of bali cattle colon (C) were 27.08% and 27.82% significantly different ($P<0.05$) higher than the nutritional value of coffee pulp without fermentation (A), respectively.

Table 2. The digestibility of dry matter, organic matter, crude protein, crude fiber, and metabolizable energy of coffee pulp with and without fermented by *Saccharomyces sp.Sb-6* isolates from bali cattle colon.

Variable	The treatment ¹⁾			SEM ²⁾
	A	B	C	
Dry matter digestibility (%)	25,64 ^b	32,58 ^a	32,77 ^a	0,25
Organic matter digestibility (%)	31,11 ^b	37,12 ^a	37,82 ^a	0,24
Crude protein digestibility (%)	62,33 ^b	66,35 ^a	66,90 ^a	0,23
Crude fibre digestibility (%)	57,20 ^b	67,07 ^a	67,25 ^a	0,29
Metabolizable energy (kcal/kg)	1756,98 ^b	2140,65 ^a	2197,01 ^a	20,23

Note:

- 1) Coffee pulp without fermented as a control (A), coffee pulp fermented by 0.20% *Saccharomyces sp.Sb-6* (B), and fermented by 0.40% *Saccharomyces sp.Sb-6* (C)
- 2) SEM: Standart Error of The Treatment Means
- 3) The different superscript at the same row is significantly different ($P<0.05$)

Organic matter digestibility of coffee pulp without fermented (A) was 31.11% (Table 2). Mean organic matter digestibility of coffee pulp fermented in treatment B and C, were 19.33% and 21.60% significantly different ($P<0.05$) higher than the control (A), respectively. Crude protein digestibility of coffee pulp without fermentation (A) was 62.33% (Table 2). Digestibility of crude protein digestibility of fermented coffee pulp with 0.20% *Saccharomyces sp.Sb-6* isolates from bali cattle colon (B), and 0.40% *Saccharomyces sp.Sb-6*

isolates from bali cattle colon (C) were 6.46% and 7.34% ($P < 0.05$) were significantly different ($P < 0.05$) higher than the control (A), respectively. Digestibility of crude fiber coffee pulp without fermentation (A) was 57.20% (Table 2). The mean digestibility of crude fiber in the fermented coffee pulp treatment B and C, were 17.26% and 17.58% significantly ($P < 0.05$) different higher than the control (A), respectively. The results showed that the content of metabolizable energy of coffee pulp without fermentation (A) was 1756.98 kcal / kg (Table 2). The average content of metabolizable energy from coffee pulp fermented in treatment B and C were 21.84% and 25.05% significantly different ($P < 0.05$) higher than the metabolizable energy content of coffee pulp without fermented (A), respectively.

DISCUSSION

Fermentation feed purpose is to improve the usability of feed and elimination of anti-nutritional substances, as well as utilize biomass formed (Utama *et al.*, 2006). Coffee pulp fermented with yeast will be able to increase the microbial biomass, so that the crude protein content of coffee pulp increased. Also reported that the fermentation process and the product are affected by the type and number of microbes, substrat types, pH, and temperature during the fermentation process. Biomass is a form of mass from the biological processes of microorganisms. Microorganisms capable of converting material into proteins. Jaelani *et al.* (2008) reported that fermented palm kernel cake can markedly increase the crude protein content of palm kernel cake compared to without fermentation. The fermentation process has the objective to produce a product (material feed) that have a nutrient content, texture, better biological availability, and reduce substance antinutrisi. Suparjo *et al.* (2003) stated that the fermentation of rice bran with 0.2% *Aspergillus Niger* cultured for 72 hours can markedly increase protein and phosphorus content of rice bran, on the contrary lower crude fiber content and acid phytat rice bran. Jaelani *et al.* (2008) reported that fermentation of feedstuffs with *Trichoderma reesei* can increase the energy content, total soluble sugars, and crude protein content. Increase the energy content of fermented coffee pulp was due to the formation of sugars derived from the breakdown of crude fiber. According Bidura *et al.* (2012), fermentation of rice bran by *Saccharomyces spp.Gb-7 and Gb-9* may increase microbial biomass, so the crude protein content of rice bran increased. The success of the fermentation process is influenced by the type and number of microbes are used, the type of substrate, pH, and temperature during fermentation. Biomass is a form of mass of the results of the biological processes of microorganisms. Microorganisms capable of converting the material into microbial protein better known as single cell protein. In the same report, fermentation process has the objective to produce a product (material feed) that have nutritional content, texture, and better biological value, as well as lowering antinutrisi substances. Utama *et al.* (2006) reported that the yeast *Saccharomyces cerevisiae* is yeast capable of producing the enzymes amylase and selulolase, so as to increase the digestibility of protein and crude fiber such as cellulose and hemicellulose, as has been overhauled in the form of a simple monosaccharide. Cellulolytic yeasts capable of producing the enzyme 1,4 beta-endo-glukonase, 1,4 beta-exo glukonase, and beta-glucosidase that can degrade components of crude fiber into soluble carbohydrates (Howard *et al.*, 2003). The longer the storage time of feed ingredients when fermented by *Trichoderma viride*, the more the loss of dry matter and organic matter material, instead increasing the crude protein content and materials decreased crude fiber content material (Sumarsih *et al.*, 2007).

Also reported that the longer the fermentation process or materials storage time, the increased dry matter digestibility and organic matter materials. The energy content of fermented coffee pulp was significantly increased compared with no fermented. As reported by Jaelani *et al.* (2008), the increase in energi content of palm kernel cake (palm kernel cake/meal) as a result of fermentation by the fungus *T. reesei* of 1824.13 kcal/kg to 1930.44 kcal/kg suspected because of the degradation of mannan polysaccharides exist in palm kernel by fungus *T. reesei* into simpler forms (monosaccharides) that produces enough energy value better than in the form of polysaccharides mannan. The same thing was reported also by Sabini *et al.* (2000) which state that the fungus *T. reesei* is able to degrade a polysaccharide mannan mannotriosa, mannobiosa, and monnosa. Fermented palm kernel cake can markedly increase the crude protein content compared to palm kernel cake without fermentation (Jaelani *et al.*, 2008). The digestibility of dry matter, organic matter, crude protein, crude fibre, and metabolizable energy were slightly increased by *Saccharomyces sp.Sb-6* isolates from Bali cattle colon fermentation. These results indicated that carbohydrates other than fibres were used for microbial growth (*Saccharomyces sp.Sb-6*) and the reduction of nitrogen free extract resulted in increased concentration of the other components. Yi *et al.* (1996) reported that supplementation of microbial in diets improved N retention in broiler chickens and *in vitro* digestibility of vegetable protein. Also, Chen *et al.* (2005) reported that addition of 0.20% complex probiotic (*L.acidophilus* and *S.cerivisae*) in basal diets were increased digestibilities of dry matter. Fermented of coffee pulp ingredient by *Saccharomyces sp.Sb-6* isolates from Bali cattle colon had better digestibilities, because *Saccharomyces sp.Sb-6* isolates from bali cattle colon in the gastrointestinal tract can part of an probiotics sources (Bidura *et al.*, 2014). Also, Piao *et al.* (1999), suggested that probiotics in the gastro intestinal tract can improve protein and energy retention on the body of birds. These fungal are effective in degrading of the complex compounds such as beta-glucans and arabinoxylans (Bedford and Classen, 1992). Chen *et al.* (2005) reported that the addition of complex 0.20% probiotic (*L. acidophilus* and *S. cerivisae*) in the diet can increase the digestibility of dry matter feed. The coffee pulp fermented by *Saccharomyces sp.Sb-6* isolates from bali cattle colon will be able to soften and break down the cell walls of coffee pulp and yeast capable of releasing microfibrils, so that the structure of the cell wall of rice bran become brittle and more open. The yeast work gradually in breaking the cell wall components. Through fibrils and hyphae yeast secrete extracellular peroxidase enzymes. The extracellular peroxidase enzymes actively working on lignolysis activity, thus breaking the bond lignocellulose and lignin fraction breaks down into CO₂. Fermentation using microbes can improve the digestibility of feed nutrients (Bidura and Suastina, 2002). *Saccharomyces sp.Sb-6* isolates from Bali cattle colon as part of probiotic could improve protein and energy retention in the gastro intestinal tract of the birds (Piao *et al.*, 1999). Cho *et al.* (2007) reported that supplementation of microbe in diet could improve the bioavailability of dietary. Wang *et al.* (2004) reported that the inclusion of fiber sources such as wheat bran or potato starch reduced the maintenance of energy requirement. These fungi are effective in degradating the complex compounds such as beta-glucans and arabinoxylans (Dubey, 2006). Chen *et al.* (2005) reported that dietary supplementation of complex probiotic slightly improved digestibility of nutrients. Inconsistent reports about the effect of probiotics may be due to several aspects such as

strains of bacteria, dose level, diet composition, feeding strategy, feed form, and interaction with other dietary feed additives. Utama (2011), reported that the administration of *S. cerevisiae* in the diet can increase the digestibility of protein and crude fiber components, such as cellulose and hemicellulose, as has been overhauled in the form of simple monosaccharide. Reported by Bidura *et al.* (2014), that the pollard fermentation by *Saccharomyces spp* isolated from colon of Bali cattle can markedly increase the digestibility of dry matter, organic matter, protein and crude fiber of pollard, and can significantly increase the metabolizable energy of pollard. The same thing was reported by Candrawati *et al.* (2014), that the use of *Saccharomyces spp* isolated from feces of Bali cattle in the process of fermentation of rice bran, can markedly increase the digestibility of dry matter, organic matter, and crude fiber of rice bran, and can significantly increase the metabolizable energy content of rice bran. Many studies have clearly demonstrated that the addition of probiotics culture or enzymes to diets rich in NSP resulted in a significant reduction of the intestinal viscosity enhances energy and protein utilization. Wang *et al.* (2004) reported that degree of microbial fermentation in the large intestine improves the bioavailability of dietary. Yi *et al.* (1996) reported that supplementation of microbial in diets improved N retention in broiler chickens and *in vitro* digestibility of vegetable protein. Also, Chen *et al.* (2005) reported that addition of 0.20% complex probiotic (*L. acidophilus* and *S. cerivisae*) in basal diets increased digestibility of DM and CP. Hong *et al.* (2004) reported that fermentation of feed using *Aspergillus oryzae* increased digestibility of its DM and CP. The inclusion of soluble dietary fiber (wheat bran) increased daily NSP excreted from feces (Wang *et al.*, 2004; Suprapti *et al.*, 2008), increased both of metabolizable energy and crude protein contents of palm kernel meal (Jaelani *et al.*, 2008). Chen *et al.* (2005) reported that dietary supplementation of complex probiotic slightly improved digestibility of nutrients. Hong *et al.* (2004) reported that feeding fermented using *Aspergillus oryzae* significantly increased dry matter digestibility and crude protein. According to Jaelani *et al.* (2008), an increase in metabolizable energy content of palm kernel cake as a result of fermentation by the fungus *T.reesei*. This is due to the degradation of mannan polysaccharides exist in palm kernel cake by the fungus *T. reesei* into a simpler form (monosaccharides) which generates enough energy value better than in the form of polysaccharides mannan. The same thing was reported also by Sabini *et al.* (2000), that the fungus *T.reesei* capable of degrading polysaccharides mannan to mannotriosa, mannobiosa, and monnosa.

CONCLUSION

We conclude that fermentation of coffee pulp by 0.20-0.40% *Saccharomyces sp.Sb-6* isolates from Bali cattle colon might improve quality of feed and increase metabolizable energy of coffee pulp as feed.

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Corresponding author: I.G.N.G. Bidura, Magister Programs of Animal Science, Faculty of Animal Science, Udayana University, Denpasar, Bali, Indonesia
Email: bidura_unud@yahoo.com