Effect of Inoculations of *Trichoderma viride* and *Saccharomyces cerevisiae* Mixed Culture on Chemical Composition, Fiber, Digestibility and *Theobromine* Cocoa Pod Fermentation

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Abstract: The objective of the study was to identify the effect of *Trichoderma viride* and *Saccharomyces cerevisiae* inoculant on chemical compositions, digestibility, and *theobromine* concentration of fermented cocoa pod. This experiment consisted of four treatments, namely cocoa pods without fermentation as control (R0); fermentation of cocoa pods with inoculant *T. viride* (R1); fermentation of cocoa pods with inoculant *S. cerevisiae* (R2); and fermentation of cocoa pods with inoculant *T. viride* and *S. cerevisiae* mixed culture (R3). Each treatment had 3 replicates, and then was fermented for 10 days. Variables observed were the chemical compositions i.e dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE), fiber fraction (Neutral detergent fiber and acid detergent fiber), *in vitro* digestibility, and *theobromine* concentration. Data were analysed by one-way analysis of variance and followed by Duncan's new multiple range test (DMRT), if there were any significant difference. Results showed the inoculum affected ($P<0.05$) the chemical composition, fiber fraction and *in vitro* digestibility. However, *theobromine* was not detected on cocoa pod without fermentation and fermentation. Compared to group R0, inoculation with *T. viride* and *S. cerevisiae* mixed culture ($P<0.05$) resulted in higher DM concentration (92.78% vs 89.72% respectively), higher CP (7.43% vs 5.63% respectively), higher NDF (79.41% vs 61.18% respectively), higher ADF (73.04% vs 47.94% respectively), but was not significantly different on DM and OM digestibility (21.22% vs 22.24%, and 22.67% vs 24.31% respectively) than cocoa pod without fermentation. It is concluded that inoculant *T. viride* and *S. cerevisiae* mixed culture increased CP concentration, but had no effect on *in vitro* digestibility.

Keywords: Cocoa pod; fermentation, *Trichoderma viride, Saccharomyces cerevisiae*

1. Introduction

Limited availability of forage makes man look for other alternative feeds as partial or total replacement. Plantation wastes to be one option in dealing with the issue of the lack of forage availability in the dry season, but their waste products could also pollute the environment. According to Ditjen Perkebunan (2012), there were approximately 1,745,789 ha of cocoa plantation in 2011. Cocoa pod contain various minerals. Toharmat *et al*. (2005), reported that feeding cocoa pod to Etawa as a fiber source also provide Mg and Zn in a higher concentration than those provided by grass, bark coffee, and rice straw. Wahyuni *et al*. (2008) reported that fermented cocoa pod could be used as animal feed in growing goats up to the level of 40%. Puastuti *et al*. (2010) reported that a ration based on cocoa pod without ammonia-

tion but with organic Zn supplementation resulted in weight gain equivalent to the daily ration of grass-based, supported by sufficient consumption rate and nutrient digestibility, N retention and good fermentation characteristics. Ginting (2004) reported that pod husks of more than 15% in the ration could reduce performances of goats.

Cocoa plantation wastes such as pod husks are commonly just piled and left to degrade in the field after being harvested. This practice could cause discomforting odor that cause disease to the cocoa plants. Pod husks contained anti nutritive substances such as *theobromine* that limits its level of inclusion in the diets. Efforts to improve the nutritive quality of the cocoa pod included, drying, addition of urea (ammoniation), and fermentation. The objective of this study was to investigate the effect of fermenting cocoa pod using different types of inoculant on the chemical composition, digestibility and thebromine concentration. The objective of the study was to identify the effect of *Trichoderma viride* and *Saccharomyces cerevisiae* inoculant on chemical compositions, digestibility, and *theobromine* concentration of fermented cocoa pod.

2. Materials and Method

The experiments were performed at the Laboratory Animal Food Technology, Laboratory of Biochemistry of Nutrition, Food Animal Nutrition Department, Faculty of Animal Science, Gadjah Mada University. Instrument is used included weights capacity 10 kg with sensitivity of 0.1 kg, sealer and vaccum, grinder with sieve diameter's 1 mm, analytic weights with sensitivity of 0.0001 g, pH meter, digital thermometer, oven, tube, high performance liquid chromatoghraphy Knauer tipe UV 6000 LP. Substance used included cocoa pod, inoculant of *L. plantarum*, Inoculant of *S. cerevisiae*, cassava meal, water, chemical for analysis proximate, chemical for parameter ruminal fermentation, chemical for *theobromine* analysis, and chemical for *in vitro* digestibility.

2.1 Substrate Preparation, Fermentation and Treatments

Fresh Cocoa pods were collected from a traditional farming Gunung Kidul Regency. Cocoa pods cut to size of 1 x 5 cm and sun-dried for 10 hours to decrease the water concentration. Two species of fungi were used as inoculants, namely *Trichoderma viride* and *Saccharomycess cerevisiae.*

Fermentation was conducted at laboratory scale in a room that was sterile. The room was watered with disinfectant, the tools were sterilised with alcohol (90%). Cocoa pods were fermented in fermentor bag (as silo), and inoculation with *T. viride* and *S.cerevisiae* was performed at 1% dry matter, by mixing and spreading the inoculant evenly. Each silo was made to be aerobic by making a hole.The length of fermentation was set at 10 days at room temperature (Nelson and Suparjo, 2011; Ginting and Krisnan, 2006).

Three processing treatments and one control treatment were as follows:

- R0: Unfermented cocoa
- R1: Cocoa pod fermentation treatment using *T. viride*
- R2: Cocoa pod fermentation treatment using *S. cerevisiae*

- R4 Cocoa pod fermentation using *T. viride* + *S. cerevisiae*

2.2 Chemical Analysis

Samples of dried silages were analyzed for their chemical compositions by proximate analyses (AOAC, 2005). Neutral Detergen Fiber (NDF) and Acid Detergen Fiber (ADF) analyses were performed according to the procedures of Van soest, (1987). *In vitro* digestibility study was performed according to the procedures of Tilley and Terry (1963) at Biochemical Nutrition Laboratory, of Animal Science Faculty, the Gadjah Mada University. Theobromine analysis to was conducted as recommended by European Food Safety Authority (2008) using HPLC (AOAC, 2005).

Data of chemical composition, fiber fraction, and *in vitro* digestibility were analyzed by analysis of variance (ANOVA) and followed by Duncan's new multiple range test (DMRT) if there were any significant differences (Steel *et al.,* 1993). All statistical calculation were performed using software of Statistical Product and Service Solution version 16.0 (Soleh, 2005).

3. Results and Discussion

3.1 Proximate and fiber fraction composition of fermented cocoa pod

The proximate compositions were shown in Table 1. The fermented cocoa pods had higher dry matter content than that of the unfermented cocoa pod. The Increased dry matter contents of the fermented cocoa pod might be caused by fungal growth in the cocoa pods. The growth of fungi was characteritically slower than that of bacterial *Trichoderma viride* secreted cellulose at day 8, and influenced dry matter concentration. Suparjo *et al.* (2009) reported that fungi would utilized the organic matter from substrate and caused losses in dry matter. Mulato and Widyatomo (2003) showed that time of fermentation could affect the dry matter concentration of substrates.

Crude protein concentrations were significantly affected by the type of inoculants. Inoculation with *T. viride* have highest effect on crude protein level. Ginting and Krisnan (2006), showed that fermentation using *Trichoderma* sp as an inoculant increased the crude protein concentration of the substrates. In the current experiment the crude protein concentration was lower when treated with *T. viride* and *S. cerevisiae* mixed culture, which could be due to any negative interaction between both of inoculant.

Fungal inoculation significantly increased crude fiber contents of the fermented cocoa pods. This higher crude fiber concentration could be caused by increased composition of cell wall, of the hyfa fungal. Utomo (2001), reported that the crude fiber was organic matter which was divided to cellulose, hemicellulose, and lignin. All of these components were found in the fungal hyfa. Cocoa pods fermentation with *T. viride* and *S. cerevisiae* mixed culture showed higher free extract nitrogen concentrations. During the stationary phase, the *T. viride* used the substrate free extract nitrogen before degrading the cellulose. Kamara*et al.* (2008) showed that glucose at early phase was used by *T. viride* for growing and then started to degrade the fiber fraction. The fiber fraction are presented in Table 2.

Tabel 1. Change chemical composition unfermented cocoa pod (R0), fermented cocoa pod were inoculated T. *viride* (R1), inoculated *S. cerevisiae* (R2), inoculated *T. viride* and *S. cerevisiae* mixed culture (R3).

Variabel	DM	OM ^{ns}	CP	EE ^{ns}	CF	NFE
R0	89,72^{a}	90,55	5,63^{a}	1,71	31,08^{a}	51,93^{d}
R1	91,94^{b}	85,30	9,57^{d}	2,41	35,99^{c}	37,11^{b}
R2	92,7^{b}	84,07	8,5^{c}	2,43	31,83^{b}	41,12^{c}
R3	92,78^{b}	84.09	7,43^{b}	2,44	40,03^{d}	34^{a}

$^{a, b, c \text{ and } d}$ *different supercripts at the same rows indicate significant difference* (*P<0,05)*
^{ns} *non significant*

Neutral detergent fiber and acid detergent fiber concentrations were significantly higher in the fermented cocoa pod compared to the unfermented ones. Fungi types affected the NDF and ADF concentration of fermented cocoa pod. Cell wall increased during the prolonged time of fermentation. This result differed to that reported by Alemawor *et al.* (2009), showing that cocoa pods fermented with *Aspergillus niger* have lower cell wall contents.

Table 2. Change fiber fraction concentration unfermented cocoa pod (R0), fermented cocoa pod were inoculated *T. viride* (R1), inoculated *S. cerevisiae* (R2), inoculated *T. viride* and *S. cerevisiae* mixed culture (R3).

Variable	*Neutral Detergent Fiber*	*Acid Detergent Fiber*
R0	61,18^{a}	47,94^{a}
R1	74,66^{b}	67,32^{b}
R2	79,36^{c}	73,93^{c}
R3	79,41^{c}	73,04^{c}

$^{a, b, c \text{ and } d}$ *different supercripts at the same rows indicate significant difference* (*P<0,05)*
^{ns} *non significant*

3.2 In vitro digestibility of cocoa pod fermentation

The effects of fermentation and type of inoculants on the *in vtiro digestibility* are shown in Table 3. *In vitro* digestibility of substrate dry matter and organic matter were significantly affected by fermentation. Unfermented cocoa pod have higher DM and OM digestibility than those fermented with *T. viride,* but were not significantly different to than those fermented using inoculant of *T. viride* and *S. cerevisiae* as a mixed culture. Utomo (2001), reported that ADF was difficult to be degraded such that it could become an inhibitor in feed. Yunus (1997) reported that factors that affected *in vitro* digestibility were composition of feed, ruminal fluid temperature, time of incubation and analysis method.

Table 3. *In vitro digestibility* of unfermented cocoa pods (R0), fermented cocoa pods inoculated with *T. viride* (R1), *S. cerevisiae* (R2), *T. viride* and *S. cerevisiae* mixed culture (R3).

Variabel	DM digestibility	OM digestibility
R0	22,24^{b}	24,31^{b}
R1	10,69^{a}	12,59^{a}
R2	11,71^{a}	13,57^{a}
R3	21,22^{ab}	22,67^{ab}

$^{a, b \text{ and } c}$ *different supercripts at the same rows indicate significant difference* (*P<0,05)*
^{ns} *non significant*

The *theobromine concentration* of fermented and unfermented cocoa pods are presented in Table 4. *Theobromine* concen-

tration of unfermented and fermented cocoa pods were not detected by HPLC.This was possibly caused by the pre-preparation procedures including sun dried for 10 hours, this process might have reduced *theobromine concentration*. Sukha (2003), showed that dried and boiled could decrease *theobromine* concentration.

Table 4. *Theobromine concentrations* of unfermented cocoa pods (R0), fermented cocoa pod inoculated with *T. viride* (R1), *S. cerevisiae* (R2), *T. viride* and *S. cerevisiae* mixed culture (R3).

Variable	*Theobromine* (ppm) ^{ns}
R0	< 0,03
R1	< 0,03
R2	< 0,03
R3	< 0,03

4. Conclusion

Inoculation with *T. viride* and *S. cerevisiae* mixed cultures in fermenting cocoa pods affected the chemical composition and fiber fraction. However it does not affect the *in vitro* digestibility of DM and OM of cocoa pod. The *theobromine* concentration of the fermented and unfermented were not detected.

References

Alemawor, F., V. P. Dzogbefia, E. O. K. Oddoye, and J. H. Oldham. (2009). Enzyme cocktail for enhacing poultry utilisation of cocoa pod husk. Sci. Res. Essay. 4 (6): 555 - 559.

Association of Official Analytical Chemist. (2005). Official Methods of Analysis. 18th Ed. The Association of Official Analytical Chemist. Washington, DC.

Astuti, M. (1980). Rancangan Percobaan. Fakultas Peternakan. Universitas Gadjah Mada. Yogyakarta.

Direktorat Jendral Perkebunan. (2011). Luas area dan produksi perkebunan seluruhIndonesia.http://ditjenbun.deptan.go.id/cigraph/index.php/viewstat/komoditiutama/4-Kakao. Diakses pada 19/09/2012.

Ginting, S. P. (2004). Tantangan dan peluang pemanfaatan pakan lokal untuk pengembangan peternakan kambing di Indonesia. Proceeding Lokakarya Nasional Kambing Potong: 61 - 77.

Ginting, S. P. and R. Krisnan. (2006). Pengaruh fermentasi menggunakan beberapa strain trichoderma dan masa inkubasi berbeda terhadap komposisi kimiawi bungkil inti sawit. Proceeding Seminar Nasional Teknologi Peternakan dan Veteriner: 939 - 944.

Kamara, D. S., S. D. Rachman, andS. Gaffar. (2008). Enzymatic degradation of cellulose from banana stalks for glucose production using cellulolytic activity of *Trichoderma viride*. Proceeding of The International Seminar on Chemistry. 692-696.

Mulato, S. and S. Widyatomo. (2003). Teknik Budidaya dan Pengolahan Hasil Tanaman Kakao. Pusat Penelitian Kopi dan Kakao Indonesia. Jember.

Puastuti, W., D. Yulistiani, and Supriyati. (2008). Ransum berbasis kulit buah kakao diperkaya mineral: tinjauan pada kecernaan dan fermentasi rumen *in vitro*. Proceeding Seminar Nasional Teknologi Peternakan dan Veteriner

442 – 448.

Sukha, A. D. (2003). The utilization of cocoa and cocoa by-product. Cocoa research unit. UWI.

Suparjo, K. G. Wiryawan, E. B. Laconi, and D. Mangunwidjaja. (2009). Perubahan komposisi kimia kulit buah kakao akibat penambahan mangan dan kalsium dalam biokonversi dengan kapang *Phanerochaetachrysosporium*. Media Peternakan 32 (3): 204 – 211

Soleh, A. Z. (2005). Ilmu Statistika Pendekatan Teoritis dan Aplikatif disertai Contoh Penggunaan SPSS. Penerbit Rakayasa Sains.Bandung.

Steel, R.G.D and J.H. Torrie. (1993). Prinsip dan Prosedur Statistika. Bambang Sumantri. Gramedia. Jakarta

Tillman, A. D., H. Hartadi, and S. Reksohadiprojo. (1998). Ilmu Makanan Ternak Dasar. Gadjah Mada University Press. Yogyakarta.

Tilley, J.M.A. and R.A. Terry. (1963). A two stage technique for in the *in vitro* digestion of forage crops. J. Grassland Soc. 18 : 104.

Toharmat, T., E. Nursasih, R. Nazilah, N. Hotimah, T. Q. Noerzihad, N. A. Sigit, and Y. Retnani. (2006). Sifat fisik pakan kaya serat dan pengaruhnya terhadap konsumsi dan kecernaan nutrien ransum kambing. Media Peternakan. 29 (3): 146 - 157.

Utomo, R. (2001). Penggunaan Jerami Padi Sebagai Pakan Basal: Suplementasi Sumber Energi dan Protein Terhadap Transit Partikel Pakan, Sintesis Protein Mikrobia, Kecernaan, dan Kinerja Sapi Potong. Disertasi. Fakultas Peternakan. Universitas Gadjah Mada. Yogyakarta.

Van Soest, P. J. (1994). Nutritional Ecology of The Ruminant. Cornell university Press. London.

Wahyuni, T. H., I. Sembiring, and W. J. Sihombing. (2008). Tape kulit buah kakao sebagai pakan kambing boerka. Jurnal Agribisnis Peternakan 4 (2): 65-68

Yunus, M. (1997). Pengaruh Umur Pemotongan Spesies Rumput terhadap Produksi Komposisi Kimia, Kecernaan *In vitro* dan *In Sacco*. Skripsi. Fakultas Pascasarjana Universitas Gadjah Mada, Yogyakarta.

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