

Effect of Mix Culture Bacteria and Fungi in Fermented Peanut Hulls-Based Feed Supplement on Physical Quality and *In Vitro* Rumen Fermentation Parameters

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ABSTRAK

Nesti DR, Baidlowi A, Fauzi A, Tjahajati I. 2020. Pengaruh kombinasi campuran bakteri dan jamur pada kualitas fisik fermentasi pakan suplemen berbasis kulit kacang terhadap kualitas fisik dan parameter fermentasi rumen secara *in vitro*. JITV 25(1): 25-31. DOI: <http://dx.doi.org/10.14334/jitv.v25i1.2079>.

Penelitian ini bertujuan untuk mengetahui pengaruh kombinasi campuran bakteri (EM4:E) dan jamur (*Trichoderma viridae*:TV) sebagai inokulan pada fermentasi pakan suplemen berbasis kulit kacang terhadap kualitas fisik dan parameter fermentasi rumen secara *in vitro*. Pakan Basal (PB) dibagi kedalam empat perlakuan, yaitu: P₀ (PB); P₁ (E:25%+TV:75%); P₂ (E:50%+TV:50%); dan P₃ (E:75%+TV:25%), setiap perlakuan menggunakan tiga kali ulangan. Pakan difermentasi selama sembilan hari secara fakultatif anaerob. Parameter yang diamati meliputi kualitas fisik pakan (warna, aroma, kenampakan jamur, dan pH) serta parameter fermentasi rumen secara *in vitro* (pH rumen, kadar protein mikrobia, dan kadar total asam lemak terbang). Data dianalisis dengan menggunakan rancangan acak lengkap pola searah (*One-way ANOVA*), jika terdapat perbedaan nyata dilanjutkan dengan uji *Kruskall-Wallis* untuk data kualitas fisik dan uji *Duncan's New Multiple Range Test* (DMRT) untuk data parameter fermentasi dalam rumen secara *in vitro*. Hasil penelitian menunjukkan bahwa kombinasi E dan TV dengan perbandingan yang berbeda tidak mempengaruhi parameter aroma dan kenampakan jamur pada parameter kualitas fisik ($P \geq 0,05$), tetapi berpengaruh nyata ($P \leq 0,05$) terhadap perubahan warna dari coklat kehitaman (1,64) pada P₀ menjadi warna kecoklatan pada P₁, P₂ dan P₃ berturut-turut: 2,44; 2,69; dan 2,80, serta penurunan pH berturut-turut: 10,67; 10,67 dan 10,67%, dari P₀. Kombinasi inokulum dengan perbandingan yang berbeda tidak memberikan pengaruh nyata ($P \geq 0,05$) terhadap parameter pH rumen, kadar protein mikrobia dan kadar total VFA. Berdasarkan hasil penelitian dapat disimpulkan bahwa fermentasi suplemen pakan berbasis kulit kacang dengan kombinasi EM₄[®] 25%:*Trichoderma viridae* 75% memberikan hasil terbaik dalam parameter kualitas fisik pakan fermentasi ditinjau dari warna serta pH dengan tidak mengganggu parameter fermentasi dalam rumen.

Kata Kunci: EM₄[®], Fermentasi Rumen secara *In Vitro*, Kulit Kacang Tanah, Kualitas Fisik, *Trichoderma viridae*

ABSTRACT

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The purpose of this research was to determine the effect of the combination of mix culture bacteria (EM4®:E) and fungi (*Trichoderma viridae*:TV) on physical quality and *in vitro* rumen fermentation parameters of peanut hull-based feed supplements. Basal feed was divided into four treatments, which were: P₀ (BF); P₁ (E:25%+TV:75%); P₂ (E:50%+TV:50%); and P₃ (E:75%+TV:25%); and each treatment had three replications. Feeds were fermented facultative anaerobically for nine days. The observed parameters were physical qualities (color, odor, fungi appearance and pH), and *in vitro* rumen fermentation parameters (rumen pH, microbial protein content, and total volatile fatty acid content). Data were analyzed using one way ANOVA design, and the significance of differences were tested using *Kruskall-Wallis* test for the physical qualities data and *Duncan's New Multiple Range Test* (DMRT) test for *in vitro* rumen fermentation parameters. Results showed that the combination of E and TV at different level did not affect odor and the fungi appearance parameters ($P \geq 0.05$), however, it significantly affected $P \leq 0.05$ color change from blackish (1.64) in P₀ to brownish in P₁, P₂ and P₃ of 2.44; 2.69; and 2.80, respectively. The pH also decreased significantly ($P \leq 0.05$) by 10.67%. Treatment also did not affect the rumen pH, microbial protein content, and total volatile fatty acid content ($P \geq 0.05$). It is concluded that the combination of the EM₄[®] 25%:*Trichoderma viridae* 75% on peanut-hull based feed supplement fermentation gives the best result on color and pH fermented feed product without affecting the rumen fermentation process.

Key Words: EM₄[®], *In Vitro* Rumen Fermentation, Peanut Hull, Physical Quality, *Trichoderma viridae*

INTRODUCTION

In Indonesia, the availability of forages as feed sources for ruminants during the dry season is very limited, so the role of agriculture-based waste or by-products was very important as an alternative feed source. One of the potential feedstuffs from agriculture wastes is the peanut hulls. According to the Kementerian Pertanian Republik Indonesia (2019), Indonesia might produce approximately 512.198 tons of peanuts. The problem of peanut hulls as a feed ingredient is that it contains relatively high crude fiber (61,99%), and low (8.74%) crude protein (Fauzi 2019), which limits its digestibility (Tillman et al. 1991). Technology intervention could be used to improve the nutrient contents of peanut hull, such as supplementation with other feedstuffs or nutrients and through the fermentation process (Natsir 2012). Feed supplements can support the development of rumen microbes and improve feed digestibility (Wina 2005; Pamungkas et al. 2008), while fermentation process might improve the feed quality, due to the involvement of microbes in digesting and fermenting fiber (cellulose, hemicellulose) and anti-nutrient compounds (Rokhmani 2005).

Trichoderma viridae is a fungus which produce cellulolytic enzyme, which degrades complex polysaccharides into simple polysaccharides and improve the fiber digestibility (Jaelani et al. 2015). Rizali et al. (2018) showed in their research that the utilization of *Trichoderma viride* on the fermentation process of palm oil midrib and leaves might decrease crude fiber content by up to 8.81%. EM4[®] is a mix culture of bacteria, consisting mainly of five genus: *Rhodopseudomonas* sp., *Lactobacillus* sp., *Streptomyces* sp., *Actinomycetes*, and *Saccharomices* sp (Casmia 2016; Santoso & Aryani 2007); Satria & Nurhasanah 2010), that capable of degrading crude fiber and improve its digestibility (Ismanto 2018). Telew et al. (2013) showed in their research that EM4 decreased crude fiber content of rice hulls up to 24%. Lokapirnasari et al. (2015) explained in their study using the combination of bacteria *Acidothermus cellulolyticus* and *Aspergillus terreus* might decrease crude fiber content on rice bran fermentation.

According to the potential of peanut-hull and the bacteria and fungi as an inoculant source for feed fermentation, this research was conducted to evaluate the effects of combinations of mix culture bacteria and fungi on the physical and fermentation characteristics of peanut hull.

MATERIALS AND METHODS

Feed preparation and formulation

Peanut hull as the main feedstuff for this research is obtained from local farmers in Kulon Progo Regency, Yogyakarta Province, Indonesia. Other feedstuffs are

obtained from PT. Hasta Karya Damai Manunggal, Boyolali Regency, Central Java, Indonesia. Peanut hull was finely ground and then mixed with other ingredients such as: palm kernel meal, copra meal, pollard, corn gluten meal, minerals, molasses and urea. The feed formulation and nutrient contents is shown in Table 1. *Trichoderma viride* was obtained from the Pusat Antar Universitas (PAU), Universitas Gadjah Mada and EM4[®] was obtained from Sleman Regency Yogyakarta Province, Indonesia.

Inoculum preparation

Trichoderma viridae (pure) was cultured using Potato Dextrose Agar (PDA) media and re-cultured using Potato Dextrose Broth (PDB) media. Cultures were stored for 5 days at 37°C incubators, then counted using a spectrophotometer on 600nm to find out the fungal cells count. The use of the fungi inoculant on this research is on the liquid state. The cell count of the solution EM4[®] was counted using a spectrophotometer on 600nm (Syauqi 2017). The use of the EM4 is prepared directly when ready to use in.

Feed supplements fermentation and treatment groups

Water content (WC) of formulated feed supplements was adjusted to 60% using a mix of water and inoculums. 2% of inoculum on the liquid state, from total water-used to produce 60% WC is mixed with subtracted water-inoculum water. Each treatment was weighed (250 g) and was fermented using 2 kg plastic bags silos. Fermented feed supplements were divided into four groups of treatments, in three replications:

P₀: Basal feed

P₁: P₀ + *Trichoderma viridae* (75%) + EM₄[®] (25%)

P₂: P₀ + *Trichoderma viridae* (50%) + EM₄[®] (50%)

P₃: P₀ + *Trichoderma viridae* (25%) + EM₄[®] (75%)

Oxygen was sucked from the silo using vacuum tools. Silos already in anaerobic conditions were then inserted with the needle in a loose plastic section that was closed with cotton in order to form a facultative anaerobic condition. The fermentation process lasted for 9 days (Mirwan 2018).

Physical quality test

The physical qualities observed in this study were color, odor, fungi appearance and pH base on (Irawati et al. 2019). The determination of the physical quality of feed supplements was conducted by 15 reliable panelists. Feed fermentation results were taken as much as 50 grams and were placed in a small cup. Tests were conducted based on the scoring and then the data obtained was transformed into a numerical scale, as follows:

Table 1. Formulation and nutrient contents of experimental feedstuffs

Feedstuff	Formulation (%)	DM (%)	CP (%)	CF (%)	EE (%)
Peanut hull ¹	50	49.63	4.37	30.9	0.29
Pollard ²	5	4.30	0.82	0.29	0.20
Palm kernel cake ²	15	12.90	2.12	1.61	1.79
Copra cake ²	12	10.32	3.31	0.82	1.35
CGF ³	10	8.60	2.38	0.67	0.20
Urea ³	1	0.86	2.88	0.00	0.00
Molasses ³	5	3.85	0.20	0.02	0.02
Minerals ³	2	1.72	0.00	0.00	0.00
Total	100	92.18	10.8	35.7	5.87

¹Fauzi (2019), ²Hartadi et al. (2017), ³NRC (2001)

DM = Dry matter, CP = Crude protein, CF = Crude fiber, EE = Ether Extract

Table 2. Laboratory analysis of nutrient contents of experimental fermented feed

Groups	DM (%)	CP (%)	CF (%)	NFE (%)
P ₀	96.52	16.12	33.48	39.45
P ₁	95.50	16.12	39.17	33.90
P ₂	95.50	15.98	38.41	34.27
P ₃	95.53	15.73	38.31	34.68

P₀=Basal feed, P₁= P₀ + *Trichoderma viride* (75%) + EM4[®] (25%), P₂= P₀ + *Trichoderma viride* (50%) + EM4[®] (50%), P₃= P₀ + *Trichoderma viride* (25%) + EM4[®] (75%), DM = Dry matter, CP = Crude protein, CF = Crude fiber, NFE = Nitrogen Free Extract

1. Color: Score 1=blackish brown; score 2=chocolate; score 3=brownish yellow; score 4=yellow.
2. Odor: Score 1=very bad; score 2=rather rotten; score 3=slightly acidic; score 4 = acid.
3. Pathogenic fungi appearance: Score 1=lots of fungi; Score 2=middle fungi; score 3=no fungi

(Plummer 1978), and VFA determination using gas chromatography (Filípek & Dvořák 2009).

Statistical analysis

The experiment was arranged in a *Completely Randomized Design* and data were analyzed using the ANOVA. Data were processed using the SPSS version 16.0 program. The significance of difference among the treatment means was tested using Kruskal-Wallis test for the physical qualities data and the Duncan's New Multiple Range Test (DMRT) test for *in vitro* rumen fermentation parameters.

Fermented feed product pH

One gram of fermented feed was diluted with nine ml of *aquadest* and pH was determined using pH Meter (AOAC 2006).

In vitro rumen fermentation

In vitro rumen fermentation using the method of Menke & Steingass (1988) as modified by Makkar et al. (1995). Samples (300 mg) were weighed and mixed with 30 ml rumen: buffer solution (1:2) (vol/vol) and incubated for 48 hours. Gas production was measured at 0; 2; 4; 6; 8; 12; 24; and 48 incubation hours. After 48 hours of incubation, samples were separated between filtrate and the supernatant by filtration. The filtrate used for rumen pH was pH Meter (Kim et al. 2019), rumen microbial protein using Lowry's method

RESULTS AND DISCUSSION

Physical quality

Effect of the combinations of EM4[®] and *Trichoderma viridae* fungi in fermented peanut hulls-based feed supplements on the physical quality and fermented product's pH is shown in Table 3.

The inoculum combination of EM4[®] and *Trichoderma viridae* fungi in fermented peanut hulls-based feed significantly changed ($P \leq 0.05$) the

fermented feed color value parameter between P₀ group (brown) and P₁, P₂ and P₃ groups (yellowish). The color change might be caused by microorganism's metabolism which degraded organic matter in the feed during the fermentation process. Microorganism's fermentation produced heat during the process that might affect the color of the fermented products. Munawaroh & Anggraini (2017) reported that the change in color of fermentation treatment was caused not only by the influence of temperature during the fermentation process but also by the type of raw material. Yuniarta & Hartatik (2015) cited from Sianipar & Simanihuruk (2009), explained in their study that the silage without inoculum might have a darker color because the living in the early phase of active aerobic respiration, degradation of organic matter produces water, CO₂ and heat and the increasing temperature might affect the color of the silage.

The addition of different level combinations of EM4[®] and *Trichoderma viridae* fungi in the peanut hulls-based fermented feed did not change the odor parameter (P≥0.05). The results showed that the average value of the odor of supplement food in each treatment has a slightly acidic interpretation. This slight acidic odor might come from substrates degradation by the microorganism. The P₁, P₂, and P₃ groups showed higher acidic odor value compared to the P₀ group. The addition of inoculum may have caused changes in carbohydrates and their derivative compounds to

alcohol, acids, and carbon dioxide. Munawaroh et al. (2015) reported in her study that during the fermentation process there is a conversion of glucose to pyruvic acid and then to lactic acid. The production of these lactic acids might cause the aroma of fermentation to become acidic.

The addition of different level combinations of EM4[®] and *Trichoderma viridae* fungi in the peanut hulls-based fermented feed did not show any differences in the pathogenic fungi appearance parameter (P≥0.05). This could happen because *Trichoderma viridae* fungi used the substrates and prevent the usage of it by other pathogenic fungi. It could be proven by the change of the color and odor parameter. Yuniarta & Hartatik (2015) reported that the *Trichoderma* fungi appearance is significantly risen linearly with the fermentation time on corn straw fermentation using *Trichoderma* fungi. *Trichoderma sp.* might suppress the growth of pathogenic fungi by the antibiosis process, competition on substrates usage with the other pathogens fungi, and its hyper-parasitic traits which will lyse other pathogens fungi's hyphae (Purwantisari & Hastuti 2009).

The addition of different level combinations of EM4[®] and *Trichoderma viridae* resulted in the differences in pH between the P₀ groups (P≤0.05) and P₁, P₂, and P₃ (Table 4). It might occur because both inoculums have the ability to degrade organic substrates and produce lactic acid that decreased the pH of the

Table 3. The effect of the combinations of EM4[®] and *Trichoderma viridae* fungi in fermented peanut hulls-based feed supplement on the physical quality and fermented product's pH

Parameter	Treatment			
	P ₀	P ₁	P ₂	P ₃
Color*	1.64 ^a	2.44 ^b	2.69 ^b	2.80 ^b
Odor ^(ns)	2.98	3.22	3.29	3.36
Fungi ^(ns)	3.00	3.00	3.00	3.00
pH*	7,5 ^a	6,7 ^b	6,7 ^b	6,7 ^b

P₀=Basal feed, P₁= P₀ + *Trichoderma viride* (75%) + EM4[®] (25%), P₂= P₀ + *Trichoderma viride* (50%) + EM4[®] (50%), P₃= P₀ + *Trichoderma viride* (25%) + EM4[®] (75%)

Table 4. The effect of the combination of EM4[®] and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement to in vitro rumen parameters

Parameter	Treatment			
	P ₀	P ₁	P ₂	P ₃
pH ^{ns}	6,76	6,74	6,77	6,73
Rumen Microbial protein (mg/100ml) ^{ns}	8,97	9,37	11,54	10,19
Total VFA ^{ns}	118,13	107,81	126,27	131,22

P₀=Basal feed, P₁= P₀ + *Trichoderma viride* (75%) + EM4[®] (25%), P₂= P₀ + *Trichoderma viride* (50%) + EM4[®] (50%), P₃= P₀ + *Trichoderma viride* (25%) + EM4[®] (75%), ^{ns}= non-significant, VFA= Volatile Fatty Acid

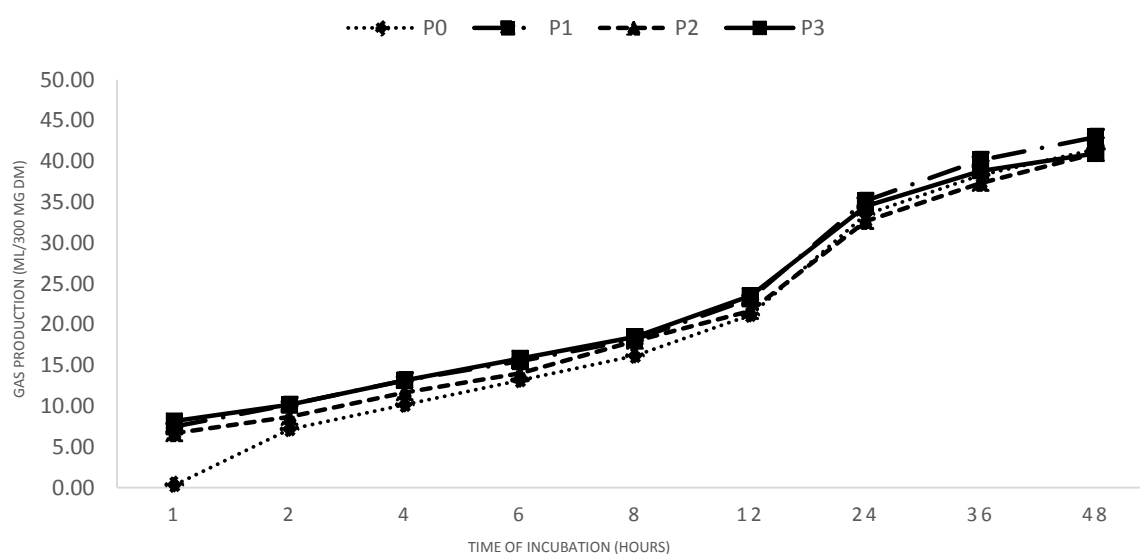


Figure 1. *In vitro* Gas production value of four different groups of the combination of EM4[®] and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement: P₀ = Basal Feed; P₁ = P₀ + *Trichoderma viridae* (75%) + EM4[®] (25%); P₂ = P₀ + *Trichoderma viridae* (50%) + EM4[®] (50%); and P₃ = P₀ + *Trichoderma viridae* (25%) + EM4[®] (75%).

fermented product. The optimal pH for the growth of *Lactobacillus* bacteria, which one of the bacteria on EM4, was 6,8 (Pramono et al. 2003) and *Trichoderma viridae* was 6,2 (Juliana et al. 2017). Zakaria et al. (2013) stated that the more colonies of lactic acid bacteria grew in the fermentation process, the fermentation process will result in lower pH. *Trichoderma viridae* fungi produce alcohol during the fermentation process while EM4[®] produced lactic acid as a by-product of the fermentation process (Umrah et al. 2009). Wahono et al. (2011) reported that in their research using EM4[®]: organic acids like acetic acid, pyruvic acid, and lactic acid decreased pH strongly, while other acids, such as butyric acid and other fatty acids had only a slight effect decrease in pH.

***In vitro* fermentation**

In vitro gas production value of four different groups of the combination of EM4[®] and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement are shown in Figure 1. Gas production illustrates the activity of microbes in degrading feed in the rumen. The results showed that incubation time-correlated with gas production and there was no significant difference ($P \geq 0.05$) between P₀, P₁, P₂ and P₃ groups on *in vitro* gas production. The gas production might not significantly different because the formulation, feed ingredients and nutritional content between the groups are slightly the same. Microbial rumen can ferment all types of feed ingredients and the role of the microbes in ruminants was very important because 65% of the main feed of ruminants is degraded

by rumen microbes (Firsoni & Lisanti 2017). Abo-Donia et al. (2014) presented in his research that fungal using *Trichoderma viridae* and urea might increase the gas production of peanut hull *in vitro* parameter because *Trichoderma* and urea might loosen the *ligno-cellulose* bonds and increase the solubility of hemicellulose and the soluble fraction.

The effect of the combination of EM4[®] and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed supplement to *in vitro* rumen parameters is showed in Table 3. The result showed that the addition of a different level combination of EM4[®] and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed did not show any differences in rumen pH after *in vitro* test ($P \geq 0.05$). The rumen pH is at normal condition indicating that the fermented feed did not affect the rumen pH condition, even though the fermented pH is slightly acidic. The pH of the feed product did not affect the rumen pH because it's on the normal range of rumen pH, which is 6.0-7.0. Mardalena (2015) stated that the normal pH rumen was a key factor for optimal rumen function. The change in rumen pH will affect the microbial population and further affect the digestibility of feed.

Rumen microbial protein content was not affected by the addition of a different level combination of EM4[®] and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed ($P \geq 0.05$). It could occur because of the supply rate of carbohydrate and nitrogen amount. The crude protein content on the fermented peanut hull feed using EM4 and *Trichoderma* fungi might play the role of the rumen microbial protein content. Abo-Donia et al. (2014) explained that

fermentation using fungi treatment might decrease NH₃-N release, and carbohydrate was degraded to provide energy for microbial multiplication resulting microbial mass and N-balance increase.

Total VFA content was not affected by the addition of different level combinations of EM4[®] and *Trichoderma viridae* fungi in the fermented peanut hulls-based feed ($P \geq 0.05$). McDonald et al. (2002) stated that the average normal VFA production was 70-150 mM, while in this study the total VFA produced ranged from 107.79 to 135.30 mM. It may be related to a similar amount of complex carbohydrate and protein substrates from fermented feed used by rumen microbes in rumen fermentation. Nurjana et al. (2015) explained that the ensiling process using *Trichoderma reesei* on napier grass did not affect the VFA content due to the high content of low digestible saccharides and crude protein in the napier grass fermented product.

CONCLUSION

The inoculum combination of EM4[®] and *Trichoderma viridae* fungi in fermented peanut hulls-based feed supplement gives accepted physical quality of fermented feed product. Color, odor, and fungi appearance met the requirements of good fermented feed. It also gives no negative effect on *in vitro* rumen fermentation parameters such as pH, rumen microbial protein content and total VFA content. However the combination of *Trichoderma viridae* (75%) + EM4[®] (25%) gives the best result in color and pH parameters while it does not affect the rumen pH, rumen microbial and VFA parameters.

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