

Feeding *Aspergillus oryzae* Fermentation Culture (AOFC) to Growing Sheep: 1. The Effect of AOFC on Rumen Fermentation

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ABSTRAK

LUBIS, D., ELIZABETH WINA, BUDI HARYANTO dan T. SUHARGIYANTATMO. 2002. Pemberian kultur fermentasi *Aspergillus oryzae* (AOFC) pada domba masa pertumbuhan: 1. Efek AOFC pada fermentasi rumen. *JITV* 7(3): 155-161.

Kultur jamur, khususnya AOFC dan *Aspergillus oryzae*, telah menjadi perhatian ahli nutrisi ternak untuk meningkatkan efisiensi penggunaan ransum. Makalah ini melaporkan studi *in vivo* penggunaan kultur fermentasi *Aspergillus oryzae* dalam ransum domba yang sedang bertumbuh. Onggok yang diperkaya dengan campuran mineral digunakan sebagai media pembiakan dan produksi *A. oryzae*. Konsentrat komersial (GT-03) diberikan pada 15 domba masa pertumbuhan, setelah dibubuhi AOFC sebanyak 0% (C₀/kontrol), 5% (C₁), dan 10% (C₂) dari bobot konsentrat. Cacahan rumput Raja segar digunakan sebagai ransum basal. Ketiga perlakuan dialokasikan secara acak kepada domba tersebut menurut rancangan acak kelompok dengan 5 ulangan. Penelitian berlangsung selama 14 minggu. Pengamatan pencernaan dilakukan dalam 10 hari terakhir penelitian. Analisis nutrisi dilakukan terhadap semua contoh pakan dan feses, cairan rumen diambil pada pertengahan periode penelitian. Pengukuran juga dilakukan terhadap pH, kadar amonia, dan produksi asam lemak atsiri (VFA) rumen, serta pencernaan bahan kering, bahan organik, protein kasar dan total serat (NDF). Perbedaan nilai rata-rata di antara perlakuan dianalisa menggunakan uji jarak berganda Duncan. Pemberian AOFC meningkatkan (P<0,05) pencernaan protein dari 59,6% pada domba kontrol (C₀) menjadi 65,5% pada domba yang diberi konsentrat dengan suplementasi 10% AOFC. Pola serupa juga terjadi pada pencernaan NDF, namun tidak berefek nyata pada bahan kering dan bahan organik. Pencernaan serat yang lebih tinggi dengan adanya suplementasi AOFC sejalan dengan terjadinya peningkatan populasi bakteri selulolitik rumen. Produksi VFA juga meningkat (P<0,05) pada domba yang diberi konsentrat C₂, demikian pula asam-asam individualnya, terutama asetat dan propionat. Tidak ada perbedaan nyata pada nilai pH dan kadar amonia rumen. Tampaknya AOFC lebih sesuai digunakan untuk tujuan produksi daging.

Kata kunci: *A. oryzae*, domba, pencernaan, rumen, *in vivo*

ABSTRACT

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Cultures of fungi, especially *Aspergillus oryzae*, have been of interest to animal nutritionists to increase feed efficiency. Many experiments have been done and showed positive results on rumen fermentation and productivity of ruminants. This paper reports the results of an *in vivo* study on feeding *Aspergillus oryzae*, fermentation culture (AOFC) to growing sheep. 'Onggok' (tapioca processing waste) was used as media for AO cultivation after being enriched with a mineral mixture. Commercial concentrate (GT-03) was fed to 15 growing sheep supplemented with 0% (C₀), 5% (C₁), and 10% (C₂) AOFC (w/w). Chopped fresh King grass was used as a basal diet. The 3 treatments were randomly allotted to the sheep according to randomized block design with 5 replications. The study was carried out for 14 weeks. Digestion trial was conducted in the last 10 days of experiment. All feed and fecal samples were analyzed for nutrients. Rumen fluid was sampled at the mid experimental period. Analyses were done on rumen pH, ammonia content, (VFA) volatile fatty acids concentration, and also total digestive tract digestibility of dry and organic matter, crude protein, and total fiber (NDF). Differences in treatment means were analyzed by Duncan's MRT. Feeding AOFC resulted in increased (P<0.05) digestibility of crude protein from 59.6% in control sheep to 65.5% in sheep fed concentrate with 10% AOFC supplementation. The same pattern also occurred for NDF, but no effect was found on dry and organic matter. Higher fiber digestibility with AOFC supplementation was in line with an increase (P<0.05) in cellulolytic bacteria population in the rumen. VFA produced also increased (P<0.05), as well as individual acids content, primarily acetate and propionate. No differences (P<0.05) were detected in rumen pH and ammonia content. It appears that AOFC is more suitable for the purpose of meat production.

Key words: *A. oryzae*, sheep, digestibility, rumen, *in vivo*

INTRODUCTION

Various efforts have been done to make ruminant animals utilize feed more efficiently. In order to reduce protein degradation and energy wasting rate in the rumen, several chemicals such as ionophores, vitamins as growth promoter for rumen bacteria, and methane inhibitors (halogenated methane analogues, sulfite, and nitrate) have been added to the diets of ruminants. Probiotics have also been used to increase nutrient digestibility and feed efficiency, primarily from fungi (*Saccharomyces cerevisiae* and *Aspergillus oryzae*) and lactic acid bacteria (certain species of lactobacilli and streptococci).

Feeding of yeast culture from a by-product of alcoholic beverages as protein supplement and rumen environment conditioner has long been used and give good results with lactating dairy cows (STACKLEY *et al.*, 1979). However, most studies conducted in using fungi cultures are concerned with digestive physiology in dairy cattle and milk production. The yeast that has been widely used is *Saccharomyces cerevisiae*, and the mould is *Aspergillus oryzae*. The use of *A. oryzae* culture could increase digestion potential of fiber as an indirect effect because of the presence of certain substances that are formed during fermentation process of *A. oryzae*, including vitamin B-complex (especially thiamine and niacin) that stimulate the growth of rumen bacteria (YOON and STERN, 1995). Total microbial population in the rumen, primarily cellulolytic bacteria, increased by supplementing fungi cultures through the feed *in vitro* (DAWSON *et al.*, 1990; NEWBOLD *et al.*, 1991) as well as *in vivo* studies (VAREL and KREIKEMEIER, 1994; NEWBOLD *et al.*, 1992; OELLERMANN *et al.*, 1990). Studies conducted by BEHARKA *et al.* (1990), and NISBET and MARTIN (1991), proved that fungi culture supplementation also caused an increase in lactate utilizing bacteria population, which in turn, will reducing rumen acidity and maintain rumen pH to normal. NEWBOLD *et al.* (1992), on the other hand, reported that protozoa population in the rumen of sheep was not changed by *A. oryzae* culture supplementation. It is clear that *A. oryzae* fermentation culture could improve rumen environment condition to be more suitable for microbial fermentation, hence can improve digestion process.

This paper reports rumen fermentation pattern and changes in fiber and nutrient digestibility in sheep as affected by the presence of *A. oryzae* fermentation culture.

MATERIALS AND METHOD

This experiment was started with preparation of *Aspergillus oryzae* fermentation culture (AOFC). The

fungus used for the study was obtained from a stock culture derived from the previous experiment (*A. oryzae* SP₆₆) that has been renewed every 3 months in mineral-enriched cooked rice media and kept in the refrigerator, as explained in LUBIS *et al.* (2000). The stock culture was then inoculated in tapioca processing waste ('onggok' flour) media for production scale. The 'onggok' flour was weighed for 5 kg and mixed with water (1 : 1), then cooked and let boiling for 30 minutes. The 'onggok' was then removed into a large plastic bucket that was previously sterilized by wiping it with 70% ethanol solution. Cooled to $\pm 60^{\circ}\text{C}$ and mixed thoroughly with mineral mix (LUBIS *et al.*, 2000) while allowing it to reach temperature between 35 to 40°C . The *A. oryzae* stock culture (1% w/w) was well mixed into the media, replaced into several sterilized plastic trays (30 x 50 x 7 cm³) of about 3 cm thickness and covered with another plastic trays. The filled trays were placed on a rack in a fermentation chamber (temperature range 26 – 30°C) for 5 days. The cultured 'onggok' was then warmed ($\pm 40^{\circ}\text{C}$) until dry, then ground and kept in clean bags. This fine material (like flour, called as AOFC) was prepared several times to meet the amount needed. It should be noted that all equipment used was cleaned and sterilized during the preparation process to avoid any contamination.

The concentrate feed used in this study was a commercial concentrate for sheep (GT-03, Indofeed Co. Ltd.) containing (registered) 16% crude protein (12% digestible protein), 4% fat (ether extractibles), 7% crude fiber, and 8% ash, having 68% TDN. Three kinds of concentrate feed were prepared for feeding trial, i.e. (C₀) concentrate GT-03 without AOFC as control diet, (C₁) GT-03 supplemented with 5% (w/w) AOFC, and (C₂) supplemented with 10% w/w AOFC. The AOFC was mixed with concentrate GT-03 by using a feed mixer at Indonesian Research Institute for Animal Production (IRIAP) in Ciawi, Bogor. Analysis showed that AOFC contains 88.72% dry matter (DM) and 23.84% crude protein in DM. Nutrient composition of King grass and concentrates used in the experiment is presented in Table 1.

Fifteen growing male 'Garut' sheep (13.7 to 21.8 kg live-weight) were kept in individual pen to test the experimental rations. All sheep were treated with a dewormer (Valbazen[®]) before the feeding trial was started. The sheep were grouped into five based on live-weight, and feeding treatments were randomly allotted according to a randomized block design with 5 replications (MONTGOMERY, 1984). Chopped King grass (± 7 cm long) used as forage and fed excessively, while concentrate was offered at 500 g h⁻¹ d⁻¹. This feeding trial was carried out for 14 weeks, from early August through the second week of November 2001 at experimental unit of IRIAP, Bogor. The concentrates

Table 1. Nutrient composition (in DM) of King grass and concentrate feeds

Nutrient	King grass	Concentrates*		
		C ₀	C ₁	C ₂
Dry matter (%)	28.40	83.41	88.02	87.39
Gross energy (Mcal/kg)	3.131	3.744	4.004	4.287
Crude protein (%)	7.97	14.27	14.99	15.16
Total fiber (% NDF)	82.52	40.35	39.40	36.81
Ash (%)	11.54	8.87	8.02	8.50

*C₀: control; C₁: supplemented with 5% AOFC; C₂: supplemented with 10% AOFC

were offered at \pm 07:00 a.m. and the forage was fed later (10:00 – 11:00 a.m.). The first two weeks of experiment was spent for adaptation period to adjust the amount of forage that can be consumed by the animals.

Drinking water was provided all day long in the experiment period. Feed refusals (both grass and concentrate) were weighed separately in the next morning before feeding time. Live-weight was determined before the experiment started, then measured once a week (every Monday morning) before the sheep was fed. In the last 10 days of the experimental period, the daily feces was collected and weighed individually in the morning. All feed and fecal samples were oven-dried and analyzed for dry matter, crude protein, gross energy, total fiber (neutral detergent fiber/NDF), and ash. At the mid experimental period (week-8), 200 ml rumen fluid samples were taken between 12:00 – 13:00 h and kept in capped dry-clean bottles. The pH of the rumen fluid samples was immediately measured using a digital pH-meter. Part of the rumen fluid samples (50 ml) were kept in sterilized tight capped tubes, then directly prepared for determination of cellulolytic bacteria population that were counted using most probable number method. The rest of the samples were kept in the freezer for further analysis of ammonia by Conway method, volatile fatty acids (acetate, propionate, butyrate, valerate) using gas chromatography. Analysis of variance was done on data of all parameters determined and differences among treatment means were analyzed by Duncan's MRT using general linear model according to Statistical Analysis Systems (SAS, 1996).

RESULTS AND DISCUSSION

In general, supplementing 5% AOFC in commercial concentrate (GT-03) give a good result on total tract fiber digestibility, but better results were obtained with 10% AOFC supplementation as indicated by higher

($P < 0.05$) fiber and crude protein digestibility, higher cellulolytic bacteria population and total volatile fatty acids (VFA) production mostly due to more acetate and propionate produced.

Rumen fermentation activity

In this experiment, rumen activity is reflected from pH value of the rumen fluid, population of fibrolytic bacteria, fiber digestibility, and VFA production. The pH values of the rumen fluid of all sheep were not different among dietary treatments and slightly below neutral value, ranged from 6.30 to 6.43 (Table 2). In normal condition, the pH value of rumen fluid ranged from 6 to 7 (VAN SOEST, 1983). Slightly low rumen fluid pH in this experiment, although still in normal range, was probably because of relative high VFA concentration in the rumen. The maximum buffering capacity of acetate, propionate, and butyrate occur at pH 4.8 – 4.9 (VAN SOEST, 1983). Another acid that could lower the rumen pH is lactic acid produced during fermentation process, which was not analyzed in the present study. VAN SOEST (1983) stated that lactic acid has a lower buffering capacity than that of the VFAs. However, the circumstance does not supports previous results (*in vitro*), as *A. oryzae* fermentation extract caused an increase in lactate utilization by one species of the rumen bacteria, *Selenomonas ruminantium*, that corrects rumen pH to neutral (NISBET and MARTIN, 1990; MARTIN and STREETER, 1994).

The effect of AOFC supplementation in this study increased ($P < 0.05$) cellulolytic bacteria population and similar results were reported by other researchers (WIEDMEIER *et al.*, 1987; NEWBOLD *et al.*, 1991 and 1992; DAWSON *et al.*, 1990). NAGARAJA and BEHARKA in 1991 (YOON and STERN, 1995) found not all rumen cellulolytic bacteria species were stimulated to grow by *A. oryzae* fermentation extract, and only positive to *Ruminococcus albus* and *Fibrobacter succinogenes*. In

the experimental results reported here, analysis was only carried out on total cellulolytic bacteria. The increased number of cellulolytic bacteria was reflected in the increased ($P<0.05$) fiber (NDF) digestibility in sheep fed with 5 and 10% AOFC supplements (Table 2).

The increased fiber digestion resulted in higher total VFA concentration in the rumen fluid of AOFC treated sheep, especially at 10% level. This is in line with results obtained by VAREL and KREIKEMEIER (1994) who fed *A. oryzae* fermentation extract to dairy cows. Supplementation of 5% AOFC in concentrate only increased propionic and valeric acids, while 10% supplementation increased all fractions of VFA compared to the control diet, primarily butyric and valeric acids. Increased in propionate and butyrate followed the increased AOFC supplementation in concentrate, but acetate and valerate were irregular. It is not entirely clear yet what factor(s) could cause such pattern. Proportionally, however, the highest molar percent of acetic acid occurred in sheep fed control diet (C_0), while molar proportion of propionate and valerate to total VFA in sheep fed concentrate with AOFC supplementation (C_1 and C_2) was higher than that of the control (Figure 1).

It is clearly presented in Figure 1 that sheep fed concentrate supplemented with 5% and 10% AOFC, the proportion of acetate to total VFA was proportionally decreased. This condition is parallel with the results of *in vitro* digestion study using King grass as a substrate incubated in sheep rumen fluid (LUBIS *et al.*, 2000). Acetate to propionate ratio values was lowered as affected by AOFC supplementation in concentrate at

5% and 10% level. This means that the amount of propionate was relatively higher and/or the amount of acetate was relatively lower in rumen fluid. This fact suggesting that the AOFC is better used for meat production rather than for milk production.

Ammonia concentration in the rumen fluid of individual sheep varied from 18.79 to 30.94 mg/dl, but no significant differences could be detected statistically in treatment means (Table 2). Various experiments concerning the use of fungi culture indicate inconsistent results in ammonia production. An experiment resulted in no significant changes (MIRANDA *et al.*, 1996), while other researchers found increased (HESSION *et al.*, 1992; WIEDMEIER *et al.*, 1987), or decreased (VAREL and KREIKEMEIER, 1994) in rumen ammonia concentration. The inconsistency was probably affected by various factors such as ration and the activity and spreading of bacteria population in the rumen. Several factors can influence each other to determine variation in rumen ammonia content. Research results proved that stimulation of bacterial growth in the rumen caused by the presence of fungi culture was frequently accompanied by increased ammonia utilization by the rumen bacteria.

Other factors that probably affect rumen ammonia content are variation in feed nitrogen (protein) availability and nitrogen recycling systems in the animal body. In most cases, decreased rumen ammonia content was not accompanied by decreased degradation and deamination rates of dietary protein, but more related to increased ammonia utilization by rumen microorganism (WILLIAMS and NEWBOLD, 1990).

Table 2. Rumen fermentation parameters in sheep fed by concentrates with or without AOFC supplementation

Parameter	Concentrate treatment*		
	C_0	C_1	C_2
Fibrolytic bacteria ($\times 10^3$)	294.8 ^a ± 218.1	470.4 ^{ab} ± 254.7	714.0 ^b ± 177.1
Rumen pH	6.30 ^a ± 0.14	6.31 ^a ± 0.28	6.43 ^a ± 0.23
Rumen ammonia (mg/dl)	22.07 ^a ± 2.18	22.41 ^a ± 1.77	24.01 ^a ± 2.59
Total VFA (mM)	46.03 ^a ± 7.73	48.55 ^a ± 3.72	57.86 ^b ± 5.59
Acetate (mM)	29.86 ^a ± 4.09	27.88 ^a ± 2.27	36.16 ^b ± 3.44
Propionate (mM)	9.80 ^a ± 2.36	11.78 ^b ± 1.12	12.89 ^b ± 0.64
Butyrate (mM)	4.69 ^a ± 0.73	5.30 ^a ± 0.92	6.97 ^b ± 0.87
Valerate (mM)	1.68 ^a ± 0.43	3.59 ^b ± 1.05	2.96 ^b ± 0.73
Acetate : Propionate	3.05	2.37	2.81

*Different superscripts in a row indicating significant difference ($P<0.05$)
 C_0 : control; C_1 : 5% AOFC supplementation; C_2 : 10% AOFC supplementation

Nutrient digestibility

Supplementation of AOFC to concentrate diets in this experiment was not followed by increased dry and organic matter digestibilities. Changed value of dry matter digestibility from 63.6% in the sheep fed control diet (C₀) to 65.7% and 66.0% in sheep fed 5% AOFC (C₁) and 10% AOFC (C₂) respectively, was not significantly different (P>0.05). The same trend also occurred in organic matter digestibility (Table 3). An increase (P<0.05) occurred in crude protein digestibility in sheep fed concentrate with 10% AOFC supplementation. Total tract fiber (NDF) digestibility also increased (P<0.05) in sheep fed concentrate with 5% and 10% AOFC supplementation.

The increased protein digestibility, however, is difficult to interpret since nitrogen analysis was done only for feed and fecal samples, while urinary nitrogen and microbial protein were not determined in the experiment. However, at least data derived from this experiment showed that AOFC supplementation

increased protein digestibility.

Increased (P<0.05) NDF digestibility by supplementation of AOFC to concentrate feed was probably because of an increase of cellulolytic bacteria population (Table 2). This result is supported by a study conducted by MIRANDA *et al.* (1996) in their *in situ* experiment using Holstein heifers fed basal diets using alfalfa and barley hay, that *A. oryzae* fermentation extract (Amaferm). They reported that NDF digestibility increased from 50.5 – 56.9% in control to 58.9 – 59.3% after 48 hours incubation. It has been reported that population of rumen microorganisms was influenced by *A. oryzae* culture, i.e. increased number of fibrolytic bacteria *in vitro* (NEWBOLD *et al.*, 1991) and *in vivo* (WIEDMEIER *et al.*, 1987; NEWBOLD *et al.*, 1992). In addition, the increase in fiber digestibility due to the influence of *A. oryzae* culture was observed not only in the rumen (CAMPOS *et al.*, 1993; GOMEZ-ALARCON *et al.*, 1990) but also in total digestive tract (AYALA *et al.*, 1992).

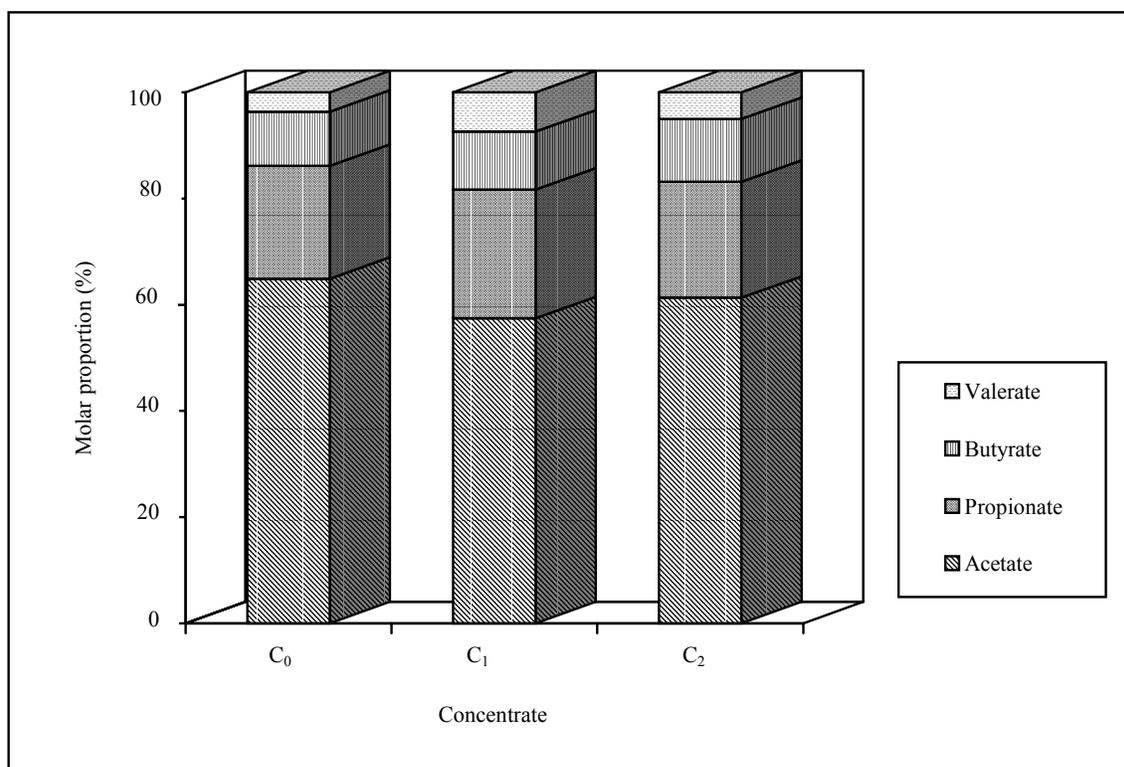


Figure 1. Molar proportion of volatile fatty acids in the rumen fluid of sheep fed by concentrate with (C₁ and C₂) or without (C₀) AOFC supplementation

Table 3. Nutrient digestibility in sheep fed with or without AOFC supplementation

Nutrient (%)	Concentrate treatment*		
	C ₀	C ₁	C ₂
Dry matter	63.6 ^a ± 4.8	65.7 ^a ± 1.5	66.0 ^a ± 1.2
Organic matter	67.1 ^a ± 4.5	68.8 ^a ± 1.8	69.3 ^a ± 1.5
Crude protein	59.6 ^a ± 4.9	61.7 ^{ab} ± 3.1	65.5 ^b ± 1.3
Total fiber (NDF)	52.7 ^a ± 2.3	57.6 ^b ± 7.7	56.5 ^b ± 2.3

*Different superscripts in a row indicate significant difference (P<0.05)

C₀: control; C₁: 5% AOFC supplementation; C₂: 10% AOFC supplementation

CONCLUSION

Rumen fermentation process in sheep was improved by supplementation of *A. oryzae* fermentation culture (AOFC) to concentrate diet up to 10% as indicated by several characteristics as follows:

1. Cellulolytic bacteria population increased one and a half time by addition of 5% AOFC and more than two fold by 10% AOFC, and followed by increase in fiber digestibility at 10% AOFC supplementation.
2. Rumen VFA production increased significantly by 10% AOFC supplementation and acetate to propionate ratio was reduced, indicating the AOFC appears more suitably used for meat production.
3. Rumen ammonia concentration and pH were not affected by AOFC supplementation.
4. AOFC can be used up to 10% in concentrate diet without negative effects.

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