Effectiveness of *Aspergillus oryzae* Fermentation Culture to Improve Digestion of Fibrous Feeds: *In vitro*

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ABSTRAK

LUBIS D, E. WINA, B. HARYANTO dan T. SUHARGIANTATMO. 2002. Efektivitas kultur fermentasi *Aspergillus oryzae* dalam memperbaiki pencernaan pakan berserat: *In vitro*. *JITV* 7(2): 90-98.

Berbagai studi yang berkaitan dengan penggunaan mikroorganisme hidup sebagai pakan tambahan telah dilakukan dalam dua dekade terakhir untuk memperbaiki proses pencernaan di dalam rumen. Di antara faktor-faktor yang mempengaruhinya adalah komposisi ransum, demikian pula status fisiologi ternaknya. Bahan-bahan yang digunakan pada percobaan ini adalah kapang *Aspergillus oryzae* (AO) yang diperoleh dari pabrik pengolahan tauco dan kecap yang terdapat di Kabupaten Bogor, Cianjur dan Sukabumi (15 sumber), juga kultur AO koleksi Balitnak (2 strain) dan satu strain dari Balitvet di Bogor. Kapang tersebut pertama kali diisolasi pada 'Potato dextrose agar' (PDA), dibiakkan pada suhu ruang (26-30^{0}C) selama 5 hari untuk pemurnian. AO dari PDA kemudian ditumbuhkan pada media beras tanak yang diperkaya dengan campuran mineral dengan cara yang sama, dikeringkan dalam oven (40-45^{0}C), lalu digiling dan disimpan di dalam lemari pendingin sebagai biang bibit untuk penggunaan selanjutnya. Untuk memproduksi lebih banyak AO (skala besar), kultur/biang tersebut dibiakkan pada 2 macam media dasar, yaitu bungkil kedele dan onggok yang juga diperkaya dengan campuran mineral, lalu dikeringkan, digiling dan disimpan di lemari pendingin sebagai kultur fermentasi *A. oryzae* atau '*A. oryzae* fermentation extract'(AOFC). Rumput Raja kering-giling digunakan untuk pengujian pencernaan *in vitro* menggunakan cairan rumen domba. Rumput tersebut tidak diberi imbuhan (sebagai kontrol) atau diimbuhi dengan AOFC dari berbagai sumber (sebanyak 10% dari berat substrat rumput Raja). Tiga AOFC yang terbaik (menurut asalnya) dari hasil pengujian pendahuluan dipilih dan digunakan untuk studi pencernaan lebih lanjut. Percobaan ini dilangsungkan menggunakan eksperimen faktorial 2 (media) x 3 (asal AOFC) berdasarkan rancangan acak lengkap dan uji jarak berganda Duncan digunakan untuk menguji perbedaan antara nilai rata-rata perlakuan. Penelitian pendahuluan menunjukkan bahwa AOFC-SP_{66}, -F_{172}, dan -TC_4 dapat digunakan dan AOFC yang terbaik adalah SP_{66}, karena meningkatkan (P<0,05) kecernaan serat (NDF) 10,5% lebih baik daripada kontrol. Tidak terdapat perbedaan (P>0,05) dalam produksi total asam lemak atsiri (VFA), namun kadar asetat dalam cairan rumen menurun (P<0,05), di lain pihak, kadar propionat dan butirat meningkat (P<0,05) akibat pemberian AOFC-SP_{66}. Kadar amonia tidak terpengaruhi oleh penambahan AOFC. Tidak terdeteksi perbedaan nyata pada parameter pencernaan antara kedua jenis media yang digunakan untuk menumbuhkan AO, dan disarankan agar menggunakan onggok untuk memproduksi AOFC dalam jumlah besar. Dari pola komposisi VFA, tampaknya AOFC lebih sesuai digunakan untuk produksi daging daripada produksi susu.

Kata kunci: Kapang, rumput Raja, kecernaan, cairan rumen

ABSTRACT

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Studies regarding the use of living microorganisms as supplement have been done in the last two decades to improve digestion process in the rumen. Many factors affect the use of the supplement, such as ration composition and physiological status of the animals. Materials used in this experiment are *Aspergillus oryzae* (AO) derived from 'tauco' (salty fermented soybean) and soybean sauce processing factories located in Bogor, Cianjur, and Sukabumi districts (15 sources), and also AO culture collections of the Indonesian Res. Inst. for Anim. Prod. (2 strains) and one strain from Indonesian Res. Inst. for Vet. Sci. in Bogor. The fungus was first isolated in potato dextrose agar (PDA), incubated at room temp. (26-30^{0}C) for 5 days for purification. The AO from PDA then inoculated into cooked rice enriched with mineral mix, oven-dried (40-45^{0}C), then ground and kept in refrigerator as a stock culture for further use. To produce more AO (scaling-up), the culture is inoculated in two basal media, i.e. soybean meal and 'onggok' (tapioca processing waste) enriched with mineral mix, dried and ground, then kept in refrigerator as *A. oryzae* fermentation culture (AOFC). Ground-dried King grass was used for *in vitro* digestion trials using sheep rumen fluid, which was not supplemented (control) or supplemented with the AOFC (10% w/w) from various sources. Three best AOFC (by origin) were chosen and used for further digestion study. The study was run using 2 (media) x 3 (AOFC origin) factorial experiment based on a completely randomized design and Duncan's MRT was applied to test differences among treatment means. Preliminary results indicated of the AOFC-SP_{66}, -F_{172}, and -CT_4 used, the best AOFC was SP_{66}, as it increased (P<0.05) fiber (NDF) digestion (10.5% better than the control). Total VFA productions were similar, but acetate content in the rumen fluid was lowered (P<0.05), while propionate and butyrate levels were alleviated (P<0.05) by the AOFC-SP_{66}. Ammonia

content was not affected by addition of AOFC. No differences were detected on digestion parameters between the two media used for AO cultivation, therefore, it is suggested to use 'onggok' for producing AOFC in large scale. VFAs composition pattern suggests that AOFC was more suitably used for meat rather than for milk production.

Key words: Fungus, King grass, digestibility, rumen fluid

INTRODUCTION

Ruminant animals naturally consume fibrous feeds like grasses and leaves or crop by products as their basal diet, due to the presence of certain microorganisms in the rumen, which developed since they started to eat grasses. Along the domestication process and direction of the value of the animals as meat or milk producer, however, concentrate feeds are also fed to some extent to maximize their production capacity commercially. The ability to digest fibrous materials highly depends on the activity of microorganisms in the rumen (VAN SOEST, 1983), which is influenced by physicochemical properties of the feed, enzymatic activity of the microbes, and micro-environment in the rumen (HUNGATE, 1966).

Many studies have been done to find which materials/feed additives can be used to optimize rumen environment to improve the digestion process. The feed additives can be enzyme, methane inhibitor, propionate enhancer, or microbial growth promoter. In the last two decades, culture of single or group of several species of living microorganisms have also been used for conditioning rumen environment suitable for certain production (milk or meat), known as probiotics. Probiotic was defined by FULLER (1989) as a feed supplement that contains living microorganisms that have beneficial effects to the animals through the improvement of microbial population balance in the digestive tract. In the United States, the word probiotic is used for living microorganism culture, enzyme prepared by microorganism, microorganism culture extract, or various combinations of them. To avoid confusion on various definition of the probiotic, the Food and Drugs Administration of the United States has suggested the producer of the supplement to not use the word probiotic, but 'direct-fed microbial' (YOON and STERN, 1995). Furthermore, YOON and STERN (1995) stated that microbial culture or extract of several kinds of microbial culture have been used through three main ways. First, it is used as an additive for preparation of silage, haylage or for hay preservation. Second, it is used for substitution or to reduce the use of antibiotics to avoid stress in cattle. Third, its utilization as a promoter to increase milk production, feed efficiency, and live-weight gain of beef cattle.

Microorganisms that have been studied and used commercially as feed supplement or additive for ruminant diets are several kinds of bacteria (primarily lactic acid bacteria such as *Lactobacillus spp*.), and fungi (especially *Saccharomyces serevisiae* and *Aspergillus oryzae*). The use of fungal culture in ruminant diets to improve animal health and productivity has recently been of interest. However, most studies done were related to dairy cattle that covering milk production and its quality. Not so many researches done on beef cattle, and only a few with sheep or goats. Experimental reports varied according to physiological status of the animals, feed components offered, and microbial species used, but mostly reported positive effects with lactating dairy cows (HUBER *et al*., 1986; KELLEMS *et al*., 1990), increased feed consumption and daily weight-gain on calves and steers (BEHARKA *et al*., 1991). The positive effect of feeding *A. oryzae* fermentation culture on live-weight gain was more realized on high concentrate diet (HUBER *et al*., 1985).

This study was aimed to develop a supplemental feed to improve rumen fermentation made of *A. oryzae* culture from several sources and a simple media could be used for its production.

MATERIALS AND METHOD

Isolates of *Aspergillus oryzae* were obtained from soy sauce and 'tauco' processing companies located in Bogor, Cianjur, and Sukabumi, and also the culture collection of Indonesian Research Institute for Animal Production (Balitnak) and Indonesian Research Institute for Veterinary Science (Balitvet) in Bogor. Fungi cultures that originated from 'tauco' were taken from 6 processing companies in Cianjur (TC_1 to TC_6) and 3 from Sukabumi district (TS_1 to TS_3), while those from soy sauce were taken from 4 processing companies in Bogor (KB_1 to KB_4), and one from Cianjur (KC_1) and Sukabumi (KS_1) districts, respectively. Three other isolates used in this study were 2 from Balitnak (F_{172} and SP_{66}) and one from Balitvet (BLV) collection. This study was conducted from June through October 2000 in the nutrition laboratory of Balitnak.

Table 1. Mineral composition (%) added to basal media used to incubate *A. oryzae*

Minerals	Basal Media	
	Soybean meal	'Onggok' flour
ZA fertilizer	5.250	5.250
Urea	1.400	3.340
Sodium hypophosphate	1.434	1.434
Magnesium sulfate	0.416	0.416
Potassium chloride	0.126	0.126
Ferro sulfate	0.062	0.080
Calcium chloride	0.056	0.075

All samples were incubated on Potato Dextrose Agar (PDA) in Petri dish at room temperature (26 – 30^0C) for 5 days. Then, each *A. oryzae* isolate was removed and incubated in boiled rice enriched with minerals mixture (Table 1) as a media placed in sterilized trays. After incubation, the media with cultures were dried at 40^0C and ground, then kept in refrigerator for later use as *A. oryzae* inoculum. The *A. oryzae* isolates used for *in vitro* digestion trials were cultured in two media, i.e. soybean meal and 'onggok' flour (tapioca processing waste) enriched with mineral mix as used for boiled rice. The 'onggok' flour and soybean meal were mixed with water (1 : 1 w/w) and boiled for 30 minutes. The media were then cooled to about 40^0C, and mixed with the mineral mix. The inoculum was put onto soybean meal and the 'onggok' media at 10% of the weight of the media and mixed thoroughly, then placed in sterilized plastic trays of approximately 2 – 3 cm thick. After incubated at room temperature for 5 days, the whole media with the *A. oryzae* culture was dried at 40^0C and ground. The material named *A. oryzae* fermentation culture (AOFC), to be used in a preliminary test for *in vitro* digestion of King grass (*Pennisetum purpuphoides*), to screen the best AOFC for later use in further examination

Ground-dried King grass samples were weighed (0.5 gram each), placed in Erlenmeyer flasks (250 ml vol.) and added with the AOFC at 10% level. *In vitro* fiber digestion test for 48 hours incubation using sheep rumen fluid was conducted according to Tilley and Terry method. Out of 18 *A. oryzae* isolates, only 3 best isolates that gave highest fiber (NDF) digestibility values were used for further testing. The King grass samples were randomly mixed with the 3 best AOFCs cultured in two different media with three replicates applying a 3 x 2 factorial experiment based on a completely randomized design (MONTGOMERY, 1984).

The *in vitro* digestion was carried out at 12, 24, and 48 hours incubation time in an incubator with an automatic shaker. In addition, samples were taken for ammonia production at 12 and 24 h incubation using Conway method, and volatile fatty acids (VFAs) content at 12 hours incubation were determined by gas chromatography. All data collected were statistically analyzed by Duncan's MRT using general linear model according to Statistical Analysis Systems procedure (SAS, 1996).

RESULTS AND DISCUSSION

The effect of addition of *A. oryzae* fermentation culture (AOFC) on fiber (NDF) digestibility of ground-dried King grass was varied according to the source of the fungus, ranging from 41.45% to 64.72% (Table 2). In general, *A. oryzae* originated from 'tauco' processing was better than that of soybean sauce processing. The highest fiber digestibility was of the King grass added with AOFC from Balitnak (SP_{66} and F_{172}), followed by AOFC isolated from one 'tauco' producer in Cianjur, i.e. AOFC-TC_4. For the AOFC originated from soybean sauce producers, only one isolate showed good result, that is from Sukabumi district (KS_1), having an NDF digestibility of 60.15%. Other AOFCs that showed fairly good results were TS_1 and TS_3, with an NDF digestibility of 61.19% and 61.39%, respectively. Based on the results and available facilities in the laboratory, three AOFCs were chosen (according to origin) for further testing, i.e. AOFC-SP_{66}, -F_{172}, and -TC_4.

Large variation in NDF digestibility as a result of adding AOFC from different origins is probably related to different strains of *A. oryzae*, which was not determined in this study. The different effects of fungi strains on growth of certain rumen bacteria has been reported by DAWSON *et al*. (1990) as well as NISBET and MARTIN (1991), who stated that the stimulation mechanism of fungi on a group of bacteria is different with that on the other group. As reported by BEHARKA and NAGARAYA in 1991 (in YOON and STERN, 1995), *A. oryzae* fermentation extract could stimulate the growth rate of certain fibrolytic rumen bacteria, *Ruminococcus albus* and *Fibrobacter succinogenes*.

Further examination on *in vitro* digestion also showed variation in parameters observed according to the source (origin) of *A. oryzae*, but there was a tendency that *A. oryzae* SP_{66} gave better results compared to the other *A. oryzae*. Analysis of variance indicated significant effects of different source of *A. oryzae* culture on most digestion parameters (Table 3), but not between the two basal media used for cultivation of the fungus (M), nor in the interaction of media and fungus origin (F x M), as a significant effect ($P<0.05$) was only found in propionate (for M) and total VFA (for F x M) production at 12 hours incubation.

The use of *A. oryzae* SP_{66} was best on fiber digestibility at 48 hours incubation, as well as on the production of total VFA, propionate, butyrate, and ammonia. In further examination, only *A. oryzae* SP_{66} could increase ($P<0.05$) fiber digestibility compared to control substrate, while *A. oryzae* TC_4 addition gave similar results with control, and *A. oryzae* F_{172} addition resulted in lower digestibility ($P<0.05$) than the control (Table 4). Digestibility of NDF in the substrate added with AOFC-SP_{66} was higher between 12 and 24 hours incubation, thereafter the increase in digestion was relatively similar till 48 hours incubation, resulted the highest digestion rate was reached with AOFC-SP_{66} treatment (Figure 1).

Table 2. Digestion rate (%, 48 h) of total fiber (NDF) in King grass added with AOFC of different sources

Source of *A. oryzae*	% NDF digested	Source of *A. oryzae*	% NDF digested
TC_1	57.89	KB_1	52.49
TC_2	50.28	KB_2	58.14
TC_3	57.45	KB_3	55.83
TC_4	61.95	KB_4	47.00
TC_5	57.59	KC_1	55.86
TC_6	41.45	KS_1	60.15
TS_1	61.19	SP_{66}	64.72
TS_2	53.82	F_{172}	62.04
TS_3	61.39	BLV	59.56
Control	60.07	-	-

Table 3. Significance level (P) of the analysis of variance for origin of fungus, cultivation media and their interaction effects on digestion parameters

Digestion parameter	Fungus origin	Basal media	Fungus x Media
NDF digestibility, 12 h	0.0376	0.3273	0.3991
NDF digestibility, 24 h	0.0015	0.2381	0.4756
NDF digestibility, 48 h	0.0016	0.4989	0.1085
Total VFA production, 12 h	0.0305	0.0614	0.0485
Acetate	0.0001	0.0528	0.6563
Propionate	0.0370	0.0380	0.3378
Butyrate	0.0034	0.1480	0.0943
Ammonia production, 12 h	0.0493	0.0825	0.3368
Ammonia production, 24 h	0.6814	0.8089	0.5733

The tendency of fiber digestibility increase was in accordance with that found by HA *et al*. (2001) with reference to relative contribution and interaction between rumen fungi and cellulolytic bacteria, where the rumen fungus (*Orpinomyces joyonii* SG_4) resulted in highest fiber digestibility at early incubation period until 2 days, then becomes weaker up to six days incubation time as compared to cellulolytic bacteria (*Ruminococcus albus* B_{199}, *R. flavifaciens* FD_1 and *Fibrobacter succinogenes* S_{85}) in *in vitro* digestion of rice straw. *In situ* experiment conducted by MIRANDA *et al*. (1996) with heifers fed alfalfa hay and barley showed that addition of *A. oryzae* fermentation extract ('Amaferm') could increase NDF digestibility from 50.5 – 56.9% in control diet up to 58.9 – 59.3%.

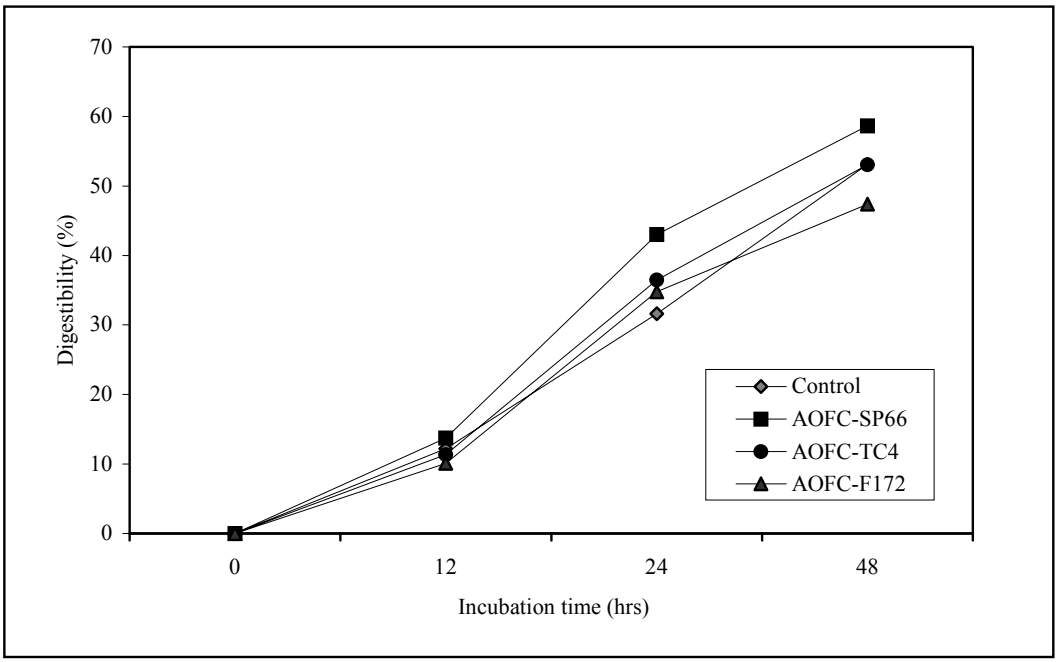

Figure 1. Fiber (NDF) digestibility of King grass with and without addition of *A. oryzae* fermentation culture (AOFC)

Table 4. *In vitro* rumen digestion parameters of King grass supplemented with *A. oryzae* of different origin

Digestion parameter	Origin of *A. oryzae**			
	F_{172}	TC_4	SP_{66}	Control
NDF digestibility, 12 h inc. (%)	$10.07^{a}\pm 0.67$	$11.35^{ab}\pm 1.91$	$13.71^{b}\pm 2.61$	$12.22^{ab}\pm 0.27$
NDF digestibility, 24 h inc. (%)	$34.76^{a}\pm 2.02$	$36.48^{a}\pm 5.47$	$43.02^{b}\pm 2.95$	$31.62^{a}\pm 0.34$
NDF digestibility, 48 h inc. (%)	$47.39^{a}\pm 2.75$	$53.07^{b}\pm 3.55$	$58.63^{c}\pm 4.01$	$53.08^{b}\pm 0.49$
Total VFA, 12 h inc. (mg/ml)	$2.08^{a}\pm 0.29$	$2.40^{ab}\pm 0.18$	$2.65^{b}\pm 0.26$	$2.28^{ab}\pm 0.28$
Acetate (mg/ml)	$0.49^{a}\pm 0.14$	$0.69^{ab}\pm 0.17$	$0.80^{b}\pm 0.18$	$1.55^{c}\pm 0.09$
Propionate (mg/ml)	$0.45^{b}\pm 0.13$	$0.34^{ab}\pm 0.08$	$0.49^{b}\pm 0.22$	$0.23^{a}\pm 0.05$
Butyrate (mg/ml)	$0.24^{ab}\pm 0.06$	$0.17^{a}\pm 0.03$	$0.39^{b}\pm 0.14$	$0.15^{a}\pm 0.07$
Other VFAs (mg/ml)	$0.90^{b}\pm 0.17$	$1.20^{b}\pm 0.15$	$0.97^{b}\pm 0.08$	$0.35^{a}\pm 0.06$
Ammonia, 12 h inc. (mM)	$23.53^{b}\pm 1.57$	$15.68^{a}\pm 3.26$	$25.77^{b}\pm 2.44$	$22.25^{b}\pm 1.41$
Ammonia, 24 h inc. (mM)	$24.59^{a}\pm 1.69$	$23.92^{a}\pm 4.55$	$26.02^{a}\pm 2.84$	$21.20^{a}\pm 1.42$

*Numbers with different superscripts in a row indicating significant difference ($P<0.05$)

Cultivation media (soybean meal and 'onggok' flour) used for *A. oryzae* cultivation in this experiment did not affect (P>0.05) fiber digestibility. This was probably because the mineral mix added to the media was sufficient to meet the fungus requirement, while more urea was added to the 'onggok' flour to supply nitrogen. Acetate production declined (P<0.05) with the addition of AOFC of all sources to the substrate, but significant increase occurred in propionate, butyrate, and other VFAs (valerate and iso-acids). This pattern resulted in no significant differences in total VFA production between control and the three AOFCs treated substrate (Table 4).

AOFC-F_{172} supplementation resulted in lowest total VFA production and its fractions. The molar proportion of individual acid in the rumen fluid based on the origin of the AOFC (Figure 2) suggested the AOFC is better used for fattening. MCDONALD *et al*. (1982) stated that more propionic and butyric in the rumen resulted in more efficient energy utilization for fattening.

Apparently, rumen microbes digest fiber and other carbohydrates to volatile fatty acids, which will be used as an energy source. Differences in the proportion of the acids also indicated that the production of volatile fatty acids is not only depend on the population of rumen microbes, but also determined by dietary compounds fed to animals, primarily carbohydrate source compounds, both structural and nonstructural carbohydrates. The structural carbohydrate will result more acetate, while nonstructural carbohydrate will give more propionate and butyrate (VAN SOEST, 1983).

However, some experimental results indicated somewhat differences in the effect of feeding fungal culture on VFA production. Several researchers found an increase in VFA production due to addition of fungal culture (VAREL and KREIKEMEIER, 1994; NISBET and MARTIN, 1991), but others found no significant effect (MIRANDA *et al*., 1996; HIGGINBOTHAM *et al*., 1994; CATON *et al*., 1993).

Refer to the experimental results reported, it seems that the increase or decrease of VFA production was related to the ingredients of the ration fed to the animals. Significant differences (P<0.05) in propionate and butyrate concentrations appeared in this study when the origin (strain) of the fungus is pooled and analyzed according to different cultivation media used (soybean meal and 'onggok' flour) like appeared in Figure 3 and Table 5.

Increased concentration of propionic, butyric and other volatile fatty acids (iso-butyric, n-valeric, iso-valeric) and reduced acetate content in the rumen fluid in all substrates supplemented with AOFC of different origin resulted in reduced acetate to propionate ratio. Apparently fungi culture could alter rumen fermentation pattern, primarily an increase in total volatile fatty acids production and changed molar proportion (EDWARDS *et al*., 1991; NISBET and MARTIN, 1990; VAREL and KREIKEMEIER, 1994; HARRISON *et al*., 1988). The fermentation pattern (VFAs) indicated that *A. oryzae* supplementation appeared to be more suitable for beef or mutton rather than for milk production.

No significant effect was detected on the influence of AOFC addition on ammonia production, regardless the origin of fungus and incubation time, except for King grass supplemented with AOFC-TC_4 at 12 hours incubation where its concentration was significantly lowered (Table 4). The kinds of media used for cultivation of *A. oryzae* also did not affect (P>0.05) ammonia production. Experiments conducted on the use of fungi cultures in ruminant rations reported inconsistent results in rumen ammonia content, no significant effect (MIRANDA *et al*., 1996), increased (HESSION *et al*., 1992; WIEDMEIER *et al*., 1987), or reduced (VAREL and KREIKEMEIER, 1994).

Many factors, individually or interactively, affecting ammonia content in the rumen. Stimulation of bacterial growth with the addition of yeast culture often increased ammonia utilization by rumen bacteria. Further, ammonia concentration is influenced by different nitrogen availability in feed materials or by nitrogen recycling mechanism in the animal body. Declined rumen ammonia content in such case is not followed by decreasing in degradation and deamination rate of protein (WILLIAMS and NEWBOLD, 1990), and it seems more related to the increasing of ammonia utilization by rumen microorganisms. Ammonia uptake and its incorporation into protein in bacterial cell that was stimulated by fungi activities and their metabolites caused a decrease in rumen ammonia concentration.

In general, experiments on the utilization of fungi cultures in ruminants indicate fairly good response, but key information related to the role of fungi in increasing production of dairy and beef cattle, sheep or goat is not yet clear. Some valuable information, however, can be extracted from this study to complete information related to the effect of *A. oryzae* fermentation culture on rumen fermentation pattern.

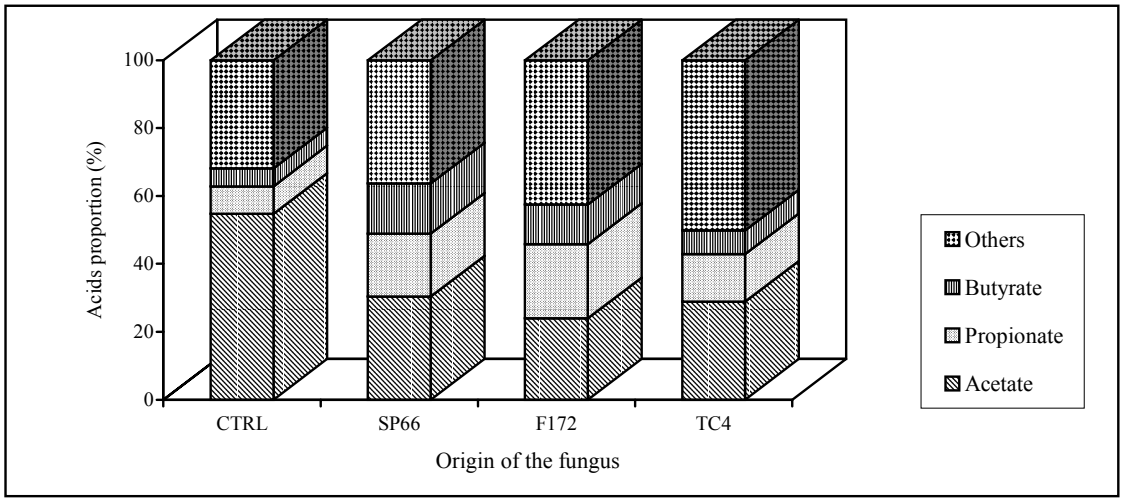

Figure 2. Volatile fatty acids proportion (%) in the rumen fluid according to *A. oryzae* origin and control

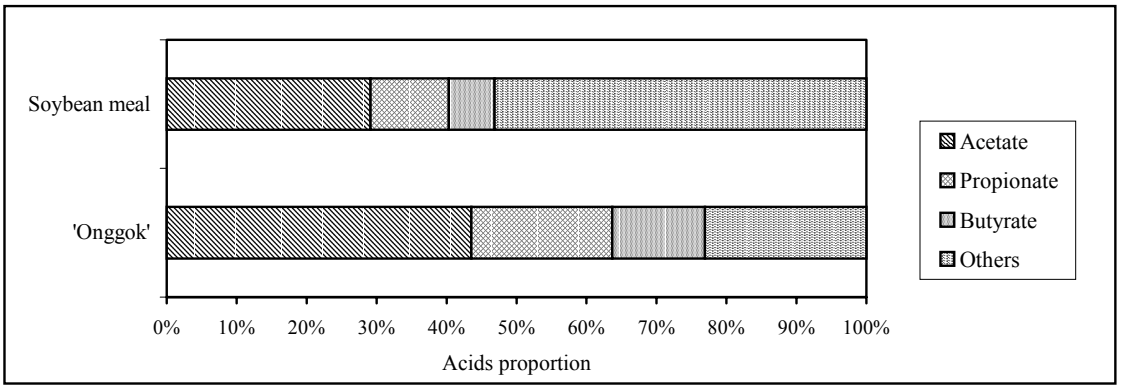

Figure 3. Volatile fatty acids proportion (%) in the rumen fluid given *A. oryzae* culture with different basal media

Table 5. *In vitro* rumen digestion parameters of King grass supplemented with *A. oryzae* of different cultivation media

Digestion parameter	Cultivation media*	
	'Onggok' flour	Soybean meal
NDF digestibility, 12 h inc. (%)	$11.43^{a} \pm 1.88$	$12.25^{a} \pm 1.09$
NDF digestibility, 24 h inc. (%)	$35.45^{a} \pm 3.93$	$37.49^{a} \pm 2.29$
NDF digestibility, 48 h inc. (%)	$52.49^{a} \pm 2.85$	$53.60^{a} \pm 3.17$
Total VFA, 12 h inc. (mg/ml)	$2.28^{a} \pm 0.60$	$2.47^{a} \pm 0.26$
Acetate (mg/ml)	$0.97^{a} \pm 0.29$	$0.80^{a} \pm 0.19$
Propionate (mg/ml)	$0.45^{b} \pm 0.37$	$0.31^{a} \pm 0.08$
Butyrate (mg/ml)	$0.30^{b} \pm 0.19$	$0.18^{a} \pm 0.04$
Other VFAs (mg/ml)	$0.56^{a} \pm 0.22$	$1.18^{b} \pm 0.17$
Ammonia, 12 h inc. (mM)	$19.30^{a} \pm 2.17$	$24.32^{a} \pm 3.49$
Ammonia, 24 h inc. (mM)	$23.58^{a} \pm 2.89$	$24.48^{a} \pm 3.48$

*Numbers with different superscripts in a row indicating significant difference ($P<0.05$)

CONCLUSIONS

1. *Aspergillus oryzae* fermentation culture (AOFC) supplementation affect rumen fermentation, but only AOFC-SP_{66} could increase fiber digestion (*in vitro*).
2. Total volatile fatty acids content in the rumen fluid was not affected by addition of AOFC. Acetate content lowered, but propionate and butyrate content increased significantly.
3. Ammonia content in rumen fluid (*in vitro*) was not significantly influenced by addition of AOFC.
4. The use of AOFC appears to be more suitable for meat than for milk producing animals.

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