

Bioconversion of Sugarcane Bagasse with Japanese *Koji* by Solid-state Fermentation and Its Effects on Nutritive Value and Preference in Goats

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ABSTRACT : The effects of 3 different strains of Japanese *koji* (*Aspergillus oryzae*, *A. sojae* and *A. awamori*) in the solid-state fermentation (SSF) of sugarcane bagasse mixed with wheat bran on chemical composition, energy, *in vivo* digestibility and preference of the fermented bagasse feeds (FBF) in goats were investigated. Diets consisted of lucerne hay cube (basal diet) and unfermented bagasse feed (control), FBF with *A. oryzae* (O), FBF with *A. sojae* (S) or FBF with *A. awamori* (A), which were mixed in a total ration of 7:3 (w/w DM). Three Nubian does were fed each of the diets, i.e. control, O, S and A in the 4 consecutive periods for digestion trials (21-day each). The goats were also used for preference trials (30-min each) of O, S and A. The O was significantly higher in CP content than others ($p < 0.05$). The crude fiber (CF), ADF and cellulose contents of control were significantly lower than those of other diets ($p < 0.05$). The S had significantly higher CF digestibility than control ($p < 0.05$), and it revealed the largest value of all. Digestibilities of NDF, ADF and cellulose in S were significantly higher than those of control (about 10, 18 and 18%, respectively, $p < 0.05$). The DE of S was significantly higher than that of others ($p < 0.05$), though there were no significant differences in DCP and TDN between control and S. The results of preference trials demonstrated that the average intake rate was not significantly different among diets, but O and S are likely to be preferable to A ($p < 0.1$). It was concluded that the SSF of bagasse feeds by Japanese *koji* can improve the fiber digestion, especially NDF, ADF or cellulose in goats, and there is a marked effect in the feed containing *A. sojae*, which may lead to the improvement of DE. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 9 : 1279-1284)

Key Words : Bagasse, Japanese *Koji*, Solid-state Fermentation, *In vivo* Digestibility, Goats

INTRODUCTION

Application of agro-industrial by-products in bioprocesses may serve a dual role in providing alternative substrates, and help to reduce environmental pollution that their disposal may otherwise cause. In the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have emerged for their utilization (Pandey et al., 2000b). Bioconversion of fibrous materials by solid-state fermentation (SSF) has received increasing interest in producing animal feeds due to its lower energy requirement, low effluent generation, direct applicability of the fermented products for feeding (Yang et al., 2001) and partly because of environmental concerns regarding the disposal of solid wastes. SSF has been used for centuries and is still used today to produce foods and also the composting of lignocellulosic fibers. Recently, the SSF has been applied to large-scale industrial processes mainly in Japan (Raimbault, 1998).

Sugarcane bagasse is a fibrous residue of sugarcane

stalks left over after the crushing and extraction of the juice. Bagasse with its low ash (2.4%) content offers numerous advantages in comparison to other crop residues such as rice straw and wheat straw which have 17.5 and 11.0% ash contents respectively, for usage in bioconversion processes using microbial cultures (Pandey et al., 2000a). In addition, bagasse can be considered as a rich solar energy reservoir due to its high yields (about 80 t/ha in comparison to about 1, 2 and 20 t/ha for wheat, other grasses and trees, respectively) and annual regeneration capacity (Pandey et al., 2000a). Although some commercial uses for the surplus bagasse have been developed, its accumulation causes a serious waste problem for the sugar industry. One potential use of the bagasse is as a feedstuff for domestic ruminants.

Filamentous fungi are the most important group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties (Raimbault, 1998). Japanese *koji* such as *Aspergillus oryzae*, *A. sojae* and *A. awamori* belong to a group of well-known and important filamentous fungi producing various traditional Japanese beverages and foods, such as *sake* (rice wine), *miso* (soybean paste), and soy sauce (Kitamoto, 2002). Several researchers reported that the use of a direct-fed microbial such as *A. oryzae* increased DM digestibility of high concentrate diets through enhanced fiber digestion (Van Horn et al., 1984; Weidmeier et al., 1987; Gomez-Alarcon et al., 1990) as well as DM intake of TMR or silage and milk production (Chiou et al., 2002). More recently, it has been suggested that the *A. awamori* not only induces a

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degradation of *shochu* distillery by-product with wheat bran and the conversion of the mixture into animal feed, but also improves fiber digestibility to some degree when adding 5 to 20% of the fermented feed to formula feed for beef cattle in Kagoshima, Japan (Yamamoto, pers. com.). Therefore, it may be expected that the *koji* would promote fiber digestion of sugarcane bagasse through the SSF mixed with wheat bran. However, little information is available on it. Moreover, there is a scarcity of studies on goats fed the fermented feed. This study was undertaken to evaluate the effects of three strains of Japanese *koji* (*A. oryzae*, *A. sojae* and *A. awamori*) in the SSF process of bagasse mixed with wheat bran on its chemical composition, energy, digestibility and preference in goats.

MATERIALS AND METHODS

Microorganisms

Spawn of *Aspergillus oryzae* and *A. sojae* was obtained from the Higuchi Matsunosuke Co., Ltd. Osaka, Japan, and that of *A. awamori* was obtained from Kirishima Highland Brewery Co., Ltd. Kagoshima, Japan. All strains were received fresh in a spore form and were used for inoculating the substrates in the SSF process.

Substrates

Dried sugarcane bagasse (SANEI Co., Ltd. Kagoshima, Japan) and wheat bran, which were on the market, were used as the substrates.

Solid-state fermentation (SSF)

Dried sugarcane bagasse was mixed with wheat bran in a ratio of 1:3 (w/w DM) in a tank bioreactor (30 kg capacity). The bioreactor was designed to allow adequate aeration, mixing of substrates and temperature controlled at 35°C. Before loading to the bioreactor, 13 kg dry weight of the substrates was mixed with water in order to adjust the moisture content at 36%. After sterilization at 115°C for 30 min and cooling to 32°C, 13 g of spawn (0.1% DM basis) was aseptically spread over the substrate and mixed well. All fermentations were conducted at 35°C for 3 days.

Digestion trial

Three Nubian does weighing 22.7±2.9 kg were kept individually in cages to determine the digestibility of the fermented bagasse feeds (FBF) and unfermented bagasse feed (UBF or control) as experimental feeds using lucerne hay cube (basal diet with known digestibility) according to the feeding standard (NRC, 1981). The FBF with *A. oryzae*, FBF with *A. sojae* and FBF with *A. awamori* were abbreviated as O, S and A, respectively. The basal diet and the experimental feed were mixed in a total ration of 7:3 (w/w DM). Water was offered *ad libitum*. Digestion trials

were carried out using the 3 goats in four consecutive periods (i.e. control, O, S and A). Each period composed of 14 days for adaptation followed by 7 days collection. Feces were totally collected daily during each period. Feces were then homogenized and weighed: 25% aliquot from daily fecal collection was oven dried for 48 h at 60°C to estimate the total fecal DM. Dry matter digestibility (DMD) of the experimental feeds was indirectly estimated from the DM intake and DM of feces excreted for the basal diet and the mixture, respectively. Dried feces were ground (1-mm screen), sampled and stored for chemical analysis.

Preference trial

The same goats as in the digestibility trial and three kinds of fermented bagasse feeds (O, S and A) were used for this study. Each animal was fed *ad libitum* between 2 of the 3 diets. Feeding period was given as 30 min for each trial. Three trials were carried out for 3 consecutive days. Dry matter intake (DMI) of each diet by goats was measured during the feeding period, and the percentage of each diet to total of 2 diets was calculated as intake rate (IR) as described by Bell (1959).

Chemical analysis

All analyses were done in triplicate. Dry matter (DM) was determined by oven drying at 60°C for 48 h. Organic matter (OM) was estimated by difference between dry matter and ash (AOAC, 1990). Chemical determination of crude protein (CP), ether extract (EE), crude fiber (CF) and ash was conducted on oven-dried feed and feces samples by the method of AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were analyzed according to Goering and Van Soest (1970) method. Gross energy (GE) content of the feed and feces samples was determined using an adiabatic bomb calorimeter (Shimadzu CA-4PJ type). Digestible energy (DE) was calculated by the subtraction of energy loss into feces from GE of the feed.

Statistical analysis

All data on chemical composition and energy concentration were subjected to ANOVA for completely randomized design. The difference in means was tested using Fisher's PLSD test performed with StatView for Windows (SAS, 1992). All data on nutritive value and intake rate were derived from the 3 goats, and the treatment means were compared with *t*-test. Statistical significance of differences was accepted at $p < 0.05$.

RESULTS

Chemical composition of materials

Bagasse had lower CP, EE and ash, and higher DM, OM,

Table 1. Chemical composition (%) of bagasse, wheat bran and their mixture (1:3 w/w DM)¹

Item	Bagasse	Wheat bran	Bagasse and wheat bran (1:3 w/w DM)
Dry matter	93.4	88.7	91.4
Organic matter	98.0	94.6	96.6
Crude protein	1.3	18.9	15.2
Ether extract	0.8	4.9	3.9
Crude fiber	43.4	9.1	13.2
Crude ash	2.0	5.4	3.4
Nitrogen free extract	52.5	61.7	64.3
Neutral detergent fiber	97.2	38.8	46.5
Acid detergent fiber	61.4	14.4	17.0
Acid detergent lignin	13.2	1.4	1.8
Hemicellulose	35.8	24.4	29.5
Cellulose	48.2	13.0	15.2
Calcium	0.03	0.1	0.1
Phosphorus	0.1	0.1	0.5
Magnesium	0.03	0.04	0.2

¹ All values except for dry matter are on DM basis.

CF, NDF, ADF, ADL, hemicellulose and cellulose, while wheat bran contained lower CF, NDF, ADF, ADL, hemicellulose and cellulose, and higher DM, OM and CP (Table 1). The addition of wheat bran into bagasse increased the CP and EE contents, while depressed the fiber components such as CF, NDF, ADF, ADL, hemicellulose and cellulose.

Changes in chemical composition during SSF

The chemical composition and energy concentration of various fermented bagasse feeds (control, O, S and A) are given in Table 2. Fermentation of bagasse feeds with Japanese *koji* significantly decreased the EE, NFE and GE contents ($p < 0.05$). The O was significantly higher in CP content compared to other diets ($p < 0.05$), while OM, ash

Table 2. Chemical composition (%) and energy concentration of various fermented (3-day) and unfermented bagasse feeds¹

Item	Diet ²				SEM
	Control	O	S	A	
Dry matter	91.4 ^a	85.7 ^d	87.7 ^b	87.0 ^c	0.1
OM	96.6	96.4	96.6	96.5	0.1
CP	15.2 ^b	16.3 ^a	15.6 ^b	15.5 ^b	0.1
EE	3.9 ^a	3.1 ^b	2.9 ^b	3.1 ^b	0.2
CF	13.2 ^b	16.4 ^a	16.7 ^a	18.1 ^a	0.6
Ash	3.4	3.6	3.4	3.5	0.1
NFE	64.3 ^a	60.7 ^b	61.3 ^b	59.8 ^b	0.7
NDF	46.5 ^b	49.7 ^{ab}	49.3 ^{ab}	50.5 ^a	0.9
ADF	17.0 ^c	21.3 ^b	19.6 ^b	24.4 ^a	0.5
ADL	1.8 ^b	2.1 ^b	2.0 ^b	2.7 ^a	0.1
Hemicellulose	29.5	28.3	29.7	26.1	1.2
Cellulose	15.2 ^c	19.2 ^b	17.6 ^b	21.7 ^a	0.5
GE (Mcal/kg DM)	4.5 ^a	4.4 ^b	4.4 ^b	4.4 ^b	1.1

^{a,b,c} Means in rows with different superscripts differ significantly ($p < 0.05$).

¹ All values except for dry matter are on DM basis.

² Control: unfermented bagasse (intact), O: bagasse fermented with *Aspergillus oryzae*, S: bagasse fermented with *A. sojae*, A: bagasse fermented with *A. awamori*.

and hemicellulose content were similar in all diets. The fiber fractions such as CF, ADF and cellulose of control were significantly lower than those of other diets ($p < 0.05$).

Nutritive value

Apparent digestibility, DCP, TDN and DE of various FBF and UFBF in goats are presented in Table 3. The digestibilities of DM, CP and hemicellulose were not significantly different among diets. The S had significantly higher CF digestibility than control ($p < 0.05$), and it showed the highest value of all. Likewise, digestibilities of NDF, ADF and cellulose in S were significantly higher than those of control ($p < 0.05$), which revealed the differences of about 10, 18 and 18%, respectively. Although there were no

Table 3. Nutritive value of various fermented (3-day) bagasse feeds and unfermented bagasse feed in goats

Item	Diet ¹			
	Control	O	S	A
Apparent digestibility (% DM)				
DM	69.4±5.7	65.2±5.8	70.8±5.2	61.4±4.4
OM	63.0±2.8 ^{ab}	58.8±2.8 ^{bc}	63.9±2.0 ^a	55.2±1.0 ^c
CP	74.3±6.9	74.5±2.0	75.9±0.5	68.1±4.0
EE	75.1±2.0 ^{ab}	79.4±4.0 ^a	70.8±3.2 ^b	61.9±5.6 ^c
CF	12.9±3.2 ^c	17.7±4.5 ^{bc}	38.2±2.4 ^a	21.8±4.3 ^b
NDF	41.4±5.2 ^b	43.3±5.5 ^{ab}	51.2±4.5 ^a	41.8±3.6 ^b
ADF	14.3±7.8 ^b	27.2±7.0 ^{ab}	32.6±4.2 ^a	23.4±9.6 ^{ab}
Hemicellulose	57.1±3.9	55.5±8.9	64.2±4.3	59.0±3.2
Cellulose	18.6±9.6 ^b	32.4±8.1 ^{ab}	37.0±3.5 ^a	26.6±9.2 ^{ab}
DCP (% DM)	11.3±1.0 ^{ab}	12.1±0.3 ^a	11.9±0.1 ^a	10.6±0.6 ^b
TDN (% DM)	64.5±2.7 ^a	59.8±2.8 ^b	64.3±1.8 ^a	55.7±0.5 ^c
DE (Mcal/kg DM)	2.8±0.1 ^b	2.6±0.1 ^{bc}	2.9±0.1 ^a	2.5±0.1 ^c

^{a,b,c} Means±SD in rows with different letters differ significantly ($p < 0.05$).

¹ See the footnotes in Table 2.

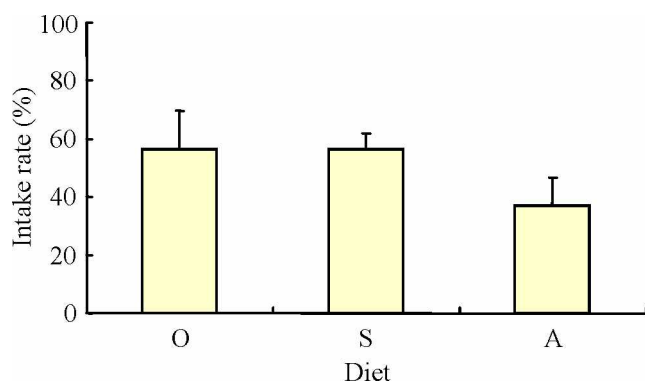


Figure 1. Intake rate (%) by goats fed bagasse based diets fermented with *Aspergillus oryzae* (O), *A. sojae* (S) and *A. awamori* (A).

significant differences in DCP and TDN between control and S. DE of S was significantly higher than that of others ($p < 0.05$).

Preference

Figure 1 shows the IR in goats fed with various FBF in the preference trial. The mean of IR was not significantly different among diets, but O and S are likely to be preferable to A ($p < 0.1$).

DISCUSSION

Results obtained in this study revealed that the mixture of the bagasse and wheat bran (1:3 w/w DM) induced an increase in CP and EE and a depression in CF, NDF, ADF, ADL, hemicellulose and cellulose. Thus, the addition of the wheat bran to the bagasse suggested a positive effect on the utilization of the low quality roughage as feed for domestic ruminants. Lallo (1996) reported that bagasse was not recommended as a sole feed for domestic ruminants because it was low in nutritive value. Therefore, it is essential to mix the bagasse with substrates such as wheat bran for improving the feeding value of animal feeds. Bradbury et al. (1956) in their report stated that the wheat bran consists of aleurone cells, which are rich in fat and proteins. The NDF and ADF contents of sugarcane bagasse in the present study were similar to the results (97.0 and 61.0%, respectively) reported by Cordova et al. (1998). On the contrary, Reddy et al. (1993) reported that NDF and ADF contents of the bagasse were 70.0 and 44.0% respectively, which was inconsistent with the present findings. The reason for this inconsistency may be due to different variety and/or maturity of sugarcane.

Vandenberghe et al. (2000) reported that protein content of cassava bagasse increased after fermentation. According to Kuhad et al. (1997), microorganisms are very attractive feedstuffs, because they can be cultivated on agro-industrial

waste, with production of large amounts of cells rich in proteins that commonly contain all the essential amino acids. In addition, Azzam (1992) used a defined mixed culture for biomass production on bagasse, and found that the growth of microorganisms was followed by the production of biomass protein. In agreement with those above mentioned, in this study it was also found that CP content of the O was significantly higher than those of others. The fact that there was higher CP content in the O than A is probably related to the result of Oyashiki et al. (1989) who found higher digestibility of protein in the *koji* prepared with *A. oryzae* than with *A. awamori*.

In the present study, fermentation of bagasse feeds with Japanese *koji* resulted in a decrease in EE and GE contents, so that those of the control were higher. This is common in the SSF process, where the microorganisms utilized part of fat in the substrate as their energy resource for their growth and reproduction (Pederson, 1999). The present study showed that the fiber fractions such as CF, NDF, ADF, ADL, hemicellulose and cellulose of O, S and A were significantly higher compared to the control. This may be due to the accumulation of acid, alkaline and/or neutral detergent insoluble substances in the SSF. As a result, fiber contents of the FBF were overestimated. Therefore, the possibility of fiber degradation by fungi during the SSF seems to be small.

There were no significant differences in the DM, OM and CP digestibility among diets in the present study. This was contrasted with other previous reports, which found that the use of a direct-fed microbial (DFM) such as *Aspergillus oryzae* increased DM digestibility through enhanced fiber digestion, and that supplementation with *A. oryzae* increased rumen and total tract digestibility of fiber fractions (Van Horn et al., 1984; Weidmeier et al., 1987; Gomez-Alarcon et al., 1990). Chen et al. (2004) also found that *A. oryzae* fermentation extract inclusion increased the DM, OM and NDF degradation rate in most forage, but it was inconsistent when tested among the by-products such like sorghum distiller's grains, bean curd pomace and soy pomace. Although there may be differences in feeding methods of microbes (direct-fed or mixing, the amount of microbes, etc.) between this study and others, the main reason for this inconsistency is not clear. As for the NDF, ADF and cellulose digestibility, there were significant positive effects of S, which was similar with the results of other previous studies.

The S with its greater digestibility of NDF, ADF and cellulose presented the highest DE of all diets, indicating the existence of various enzymes in the SSF process which result in starch and protein degradations, and subsequent increase in cellulase activity of cellulolytic microbials induced by the degradation products (mono- and

disaccharides, NPN, etc.) which helps fiber digestion in the rumen. Although the enzyme activities induced by the 3 strains of the *koji* were not identified in this study, a higher ability in NDF, ADF and cellulose digestion of the S may be due to differences in the amounts of fiber available to cellulolytic microbials in the rumen which are activated by the digestivities of starch and protein in the *koji* made with *A. oryzae*, *A. sojae* and *A. awamori* (Oyashiki et al., 1989), depending on the population of cellulolytic bacterias and their cellulase activity in the rumen.

In the present study, it was found that color and aroma were different between diets, i.e. O and S were almost similar but those were different from A. Thus, slightly higher preference of O and S diets compared to A may be derived from the different flavor. Pandey et al. (2000b) stated that the bioprocessing (SSF) of cassava bagasse was accompanied with the production of flavor and aroma compounds. Therefore, it was suggested that the SSF with *Aspergillus oryzae* and *A. sojae* tends to improve the preference via flavor and aroma.

In conclusion, the SSF of bagasse feeds (combination mixture of bagasse with wheat bran) by various Japanese *koji* (genus *Aspergillus*), improved the digestibility of some fiber components (NDF, ADF or cellulose) in the feeds. Among 3 strains of *Aspergillus*, the *A. sojae* can help fiber digestion in the FBF more efficiently compared to the others, which may reflect the improvement of DE in the feed containing *A. sojae*.

IMPLICATIONS

The present findings provide useful information on the utilization of the Japanese *koji* in the SSF process for bioconversion of agro-industrial by-product such as sugarcane bagasse from the viewpoint of increased self-sufficiency in animal feeds. This system can add value to the effects of conventional SSF process.

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