

## Effects of *Candida utilis* Treatment on the Nutrient Value of Rice Bran and the Effect of *Candida utilis* on the Degradation of Forages *In vitro*

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**ABSTRACT :** *Candida utilis* can assimilate fatty acids, so it was hypothesized that the treatment of rice by *Candida utilis* would improve feed quality by reducing fat content and adding the yeast function that would stimulate rumen microbes. In this study, the oil assimilation ability of *Candida utilis* IFO1086, 0988, 0626 and the effect of treatment of *Candida utilis* IFO1086, IFO0626 on the nutrient contents of rice bran were examined. The effect of *Candida utilis* addition on the *in vitro* degradability of forage was also investigated. It was found that the oil assimilating ability of IFO1086 and IFO0626 was significantly ( $p < 0.01$ ) higher than that of IFO0988. *Candida utilis* treatment reduced the EE content and increased the CP, ADF and NDF percentage. The absolute amount of ether extract was decreased by 35.9% in IFO1086 and IFO0626 treatment. The absolute amount of crude protein was not changed by yeast treatment. The ADF and NDF amounts were increased. The addition of *Candida utilis* increased *in vitro* forage degradability significantly ( $p < 0.05$ ). Based on these results it can be postulated that treatment of rice bran by *Candida utilis* may improve feed quality by reducing fat content, increasing the CP content and adding the function of yeast for stimulating rumen microbes. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 6 : 806-810)

**Key Words :** *Candida utilis*, Rice Bran, Degradability, Forage

### INTRODUCTION

Rice bran is the by-product of rice polishing and is used for animal feed to provide energy, protein and minerals (Moran, 1983a, b; Foster et al., 1993, 1994), but the fat content of rice bran is high, with a high degree of unsaturation (Standard Table of Feed Composition in Japan 1995; Kagawa 2002). Excess fat ingestion, especially unsaturated fat, has an adverse effect upon ruminal microbes, resulting in decreased fiber degradation and dry matter intake (Palmquist and Jenkins, 1980). On the other hand, the unsaturated fatty acids are highly susceptible to oxidation, resulting in the deterioration of rice bran quality as feed during storage (Warren and Farrell, 1990). It would be beneficial to establish a method for reducing the fat content of rice bran to avoid the adverse effect of excess fat feeding or deterioration during storage.

One genus of yeast, *Candida utilis*, can assimilate fatty acids and has been used for the treatment of waste matter from oil manufacturing plants (Zheng et al., 2001; Zheng et al., 2004). It was hypothesized that treating rice bran with *Candida utilis* would cause *Candida utilis* to proliferate and the fat content to be reduced, resulting in avoidance of the

adverse effects of extra fat.

*Saccharomyces cerevisiae* is used in bread making and brewing as well as yeast feeding to stimulate the ruminal microbes (Wiedmeier et al., 1987; Harrison et al., 1988; Dawson et al., 1990; Nisbet and Martin, 1991; Ando et al., 2004, 2005) and improve the production of ruminants (Fallon and Harte, 1987; Williams et al. 1987; Piva et al., 1993). Like *Shaccromycess cerevisiae*, *Candida utilis* feeding should stimulate the ruminal microbes and improve the production of ruminants.

The purpose of this study was to investigate the fatty acid assimilating ability and the effect upon ruminal microbes of three strains of *Candida utilis*, by as measured *in vitro* digestion of forages. Also, two of three *Candida utilis* strains were applied to rice bran to examine the effects upon nutrient content and *in vitro* the disappearance rate.

### MATERIALS AND METHODS

#### Selection and storage of yeast

*Candida utilis* strains IFO0988 (ATCC9950), IFO0626 (ATCC9256), IFO1086 (ATCC9226) were selected according to the guidance of ATCCYEASTS (1995), which indicated the possibility of fatty acid assimilation ability. The three strains of yeast were purchased from IFO (Institute for Fermentation Osaka, Osaka, Japan). Yeasts were grown on the YM-Broth solid media and stored at 4°C.

#### Measurement of oil degradation and yeast proliferation

One loopful of yeast grown on YM broth-solid plate media was placed into 100 ml YM-broth solution media.

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**Table 1.** Chemical composition of roughages

	Roughages		
	Sorghum	Italian-ryegrass	Rice-straw
Dry matter	85.0	84.3	85.8
Crude protein	6.7	16.5	6.2
ADF	17.5	17.0	32.8
NDF	28.5	38.0	54.2

These solutions were incubated for 48 h at 24°C in aerobic conditions (yeast culture). Incubation media for the measurement of oil degradation and yeast proliferation were prepared to the specifications described by Kamini et al. (2000), (yeast extract 0.5%, KH<sub>2</sub>PO<sub>4</sub> 1%, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1%). Next, 0.6 ml of yeast culture, 0.3 ml of autoclaved rice bran oil, and 30 ml of incubation medium were put into a 300-ml conical flask and aerobically incubated for 120 h at 30°C with shaking at 120 rpm. The incubation was carried out in duplicate. After incubation, 40 ml of diethyl ether was added to each flask. The flasks were well shaken and the medium was poured into a 100-ml centrifuge tube. After centrifugation, the flask was washed out by 20 ml of diethyl ether twice, and then diethyl ether was poured into the centrifuge tube. The ether portion, water-soluble portion and yeast cells were separated by centrifugation at 500G for 10 min. The ether portion was collected and poured into a weighed beaker, and diethyl ether was removed by soaking the beaker in 72°C water. The beaker was then dried at 105°C for 4 h to weigh the residual oil.

The degradability of the oil was calculated based on the amount of initial oil and residual oil. The yeast cells were washed by distilled water and centrifuged at 500 G for 10 min twice. The cells were then put on previously dried and weighed filter paper along with the aluminum tray. Yeast and filter paper along with the aluminum cane were dried at 100°C for 3 h and then dried at 135°C for 2 h. The amount of yeast cells added before incubation were measured from the incubation medium by the same manner. The amount of proliferated yeast was calculated by comparing the initial amount and residual amount of yeast.

#### ***In vitro* degradability of yeast roughage**

Yeast, Italian ryegrass, rice straw, sorghum, Italian ryegrass+yeast, rice straw+yeast, and sorghum+yeast were incubated for 24 h following the method of Tilly and Terry (1963), except that at this point, pepsin digestion was not performed. The amount of forage tested was 0.2 g, and the chemical composition of roughages is shown in Table 1. Measurements of *in vitro* degradability were carried out three times with two replications for each treatment.

#### **Yeast treatment of rice bran and measurement of nutritional value**

*Candida utilis* strains IFO0626 and 1086 were selected. About 5 g of rice bran was put into a 300-ml flask, and 40

**Table 2.** Oil degradability and proliferation rate of yeast

	Degradability (%)	Proliferation rate (times)
	IFO1086	32.8±1.23 <sup>A</sup>
IFO0986	11.5±2.12 <sup>B</sup>	17.8±1.75 <sup>B</sup>
IFO0626	33.9±1.31 <sup>A</sup>	35.0±2.36 <sup>C</sup>

<sup>A, B, C</sup> Significant in different letters (p<0.01).

**Table 3.** Chemical composition of treated and untreated rice bran (%)

	DM	EE	CP	ADF	NDF
Untreated	96.6	19.9	16.0	10.1	21.0
IFO1086	97.0	14.3	18.0	12.0	24.0
IFO0626	96.9	14.1	17.7	12.2	24.1

ml of distilled water was added and autoclaved. To that mixture, 1 ml of yeast culture was added, and as a control, 1 ml of distilled water was added. The flasks were incubated at 30°C for 120 h with reciprocal shaking at 120 rpm. All incubations were conducted twice. After incubation the treated rice bran was transferred into pre-weighed aluminum dishes and dried at 100°C for 12 h for dry matter calculation.

The ether extract contents and crude protein content were analyzed following the method of AOAC (AOAC1990). Neutral detergent fiber and acid detergent fiber were determined using the method of Van Soest et al. (1991) Yeast-treated rice bran was incubated for 24 and 72 h following the method of Tilly and Terry (1963), except that at this point, pepsin digestion was not performed.

#### **Statistical procedure**

Dunnett's multiple comparison procedure (Dunnett, 1955) was used for statistical analysis.

## **RESULTS**

#### **Oil degradation and the proliferation rate of yeast**

Table 2 shows the rate of oil degradation by yeast and the proliferation rate of yeast. Initial oil was degraded 32.8%, 11.5% and 33.9% by IFO1086, 1988 and 0626, respectively. The degradabilities of oil by IFO1086 and 0626 were significantly (p<0.01) higher than that of IFO 0988. The proliferation rate of IFO1086, IFO0988 and IFO0626 was 26.0, 17.8 and 35.0 times, respectively. Significant differences (p<0.01) were observed between the yeasts.

#### **Chemical composition of treated and untreated rice bran**

Table 3 shows the chemical composition of treated and untreated rice bran. The ether extract content was reduced by yeast treatment. In contrast the CP, ADF and NDF contents were increased by yeast treatment.

**Table 4.** Change of the amount of individual nutrients (% of initial)

	DM	EE	CP	ADF	NDF
Untreated	100.0	100.0	100.0	100.0	100.0
IFO1086	89.2 (-11.8)	64.1 (-35.9)	100.3 (0.3)	106.0 (6.0)	104.5 (4.5)
IFO0626	91.5 (-9.5)	64.1 (-35.9)	100.1 (0.1)	109.3 (9.3)	103.9 (3.9)

( ) Decrease or increase from initial amount.

**Table 5.** *In vitro* degradability of treated and untreated rice bran (%)

	24 h	72 h
Untreated	58.9±1.85 <sup>a</sup>	59.5±1.07 <sup>a</sup>
IFO1086	53.5±1.86 <sup>b</sup>	63.5±1.63 <sup>b</sup>
IFO0626	53.0±2.06 <sup>b</sup>	64.5±1.95 <sup>b</sup>

<sup>a, b</sup> Significant in different letters (p<0.05).

### Changes in the amount of individual nutrients during the yeast treatment

Table 4 shows changes in the amount of individual nutrients during the yeast treatment. The dry matter disappearance rate during IFO1086 and IFO treatment was 11.8% and 9.5%, respectively. A total of 35.9% of the ether extract disappeared following both yeast treatments. Crude protein did not disappear during yeast treatment. The amount of ADF was increased by 6.0% for IFO1086 treatment and by 9.3% for IFO 0626 treatment. Similarly, the amount of NDF was increased by 4.5% for IFO1086 treatment and by 3.5% for IFO 0626 treatment.

### *In vitro* degradability of treated and untreated rice bran

Table 5 shows the *in vitro* degradability of treated rice bran incubated for 24 h and 72 h. The degradability of untreated rice bran at 24 h was significantly (p<0.05) higher than that of treated rice bran. In contrast, the degradability of untreated rice bran at 72 h was significantly (p<0.05) lower than that of treated rice bran.

### *In vitro* degradability of yeast and forage

Table 6 shows the *in vitro* degradability of yeast. There were no significant differences between yeasts.

Table 7 shows the effect of *Candida utilis* on the *in vitro* forage degradability. The addition of *Candida utilis* increased forage degradability significantly (p<0.05), but no significant difference was observed between the yeasts.

## DISCUSSION

It was reported that *Candida utilis* can assimilate oils as

**Table 6.** *In vitro* degradability (24 h) of *Candida utilis*

Yeast	Degradability
IFO1086	70.7±3.04
IFO0988	76.1±5.05
IFO0626	70.6±2.41

<sup>a, b</sup> Significant in different letters (p<0.05).

a carbon source for proliferation (Zheng et al., 2001; Zheng et al., 2004). In this study rice oil was degraded by *Candida utilis*, and the yeast proliferated using oil as a carbon source. The results of this study support those of former reports. The oil assimilating ability and proliferation of IFO0988 was inferior to those of IFO1086 and IFO0626, so the latter two strains were selected for the yeast treatment of rice bran. A total of 35.9% of the initial fat had disappeared following treatment with the two strains of *Candida utilis*. Crude protein was not degraded during yeast treatment. The amount of ADF and NDF increased as a result of yeast treatment. Based on these results, it can be concluded that *Candida utilis* proliferated by using fatty acids as an energy source and carbon source of cell wall material or carbohydrate in cytoplasm. The main cell wall substance of yeast consists of β-glucan, mannan and chitin (Cid et al., 1995). ADF and NDF include these substances, so it can be concluded that the increase in the amount of ADF and NDF was due to the proliferation of *Candida utilis* using fatty acids. Concerning the crude protein, some nitrogenous compounds might be incorporated into the yeast cell while others might remain in the rice bran, but there was no entrance or escape of nitrogenous compounds during the treatment. The ether extract content was reduced by the *Candida utilis* treatment of rice bran, and likewise the ADF and NDF contents were increased. As for crude protein, even though the absolute amount was not changed during yeast treatment, the relative values were elevated by the decrease of ether extract content.

The addition of *Candida utilis* increased the *in vitro* degradability of roughage, which means that, much like the case with *Saccharomyces cerevisiae* (Wiedmeier et al.,

**Table 7.** Effects of yeast addition on the roughage degradation

Yeasts	Roughages			
	Sorgum	Italian-ryegrass	Rice-straw	Average
No addition	39.5±0.62	36.7±0.40	19.2±0.43	31.8 <sup>a</sup>
IFO1086	45.8±1.02	46.7±0.98	26.8±0.77	39.8 <sup>b</sup>
IFO0988	45.9±0.85	50.1±0.50	24.7±0.78	40.2 <sup>b</sup>
IFO0626	48.8±1.03	51.0±0.98	26.5±0.86	42.1 <sup>b</sup>

<sup>a, b</sup> Significant in different letters (p<0.05).

1987; Harrison et al., 1988; Dawson et al., 1990; Nisbet and Martin, 1991; Ando et al., 2004, 2005), it can be postulated that *Candida utilis* stimulate the ruminal microbes to improve the production of ruminants (Fallon and Harte 1987; Williams et al., 1987; Piva et al., 1993).

The degradability of untreated rice bran at 24 h was significantly ( $p < 0.05$ ) higher than that of treated rice bran. In contrast, the degradability of untreated rice bran at 72 h was significantly ( $p < 0.05$ ) lower than that of treated rice bran. Easily fermentable matter such as nonstructural carbohydrate was used by yeast in yeast-treated rice bran, which indicates that the degradability of treated rice bran may be lower than that of untreated rice bran at an early stage of incubation. And also higher contents of ADF and NDF in treated rice bran might cause the lower degradability. At a late stage of incubation, the degradability of structural carbohydrate might be elevated by adding yeast or reducing fat contents, in which case the degradability of treated rice bran may be higher than that of untreated rice bran. In control the degradability of rice bran was not changed at 72 h incubation compared with 24 h. This might be due to the degradability of rice bran was reached to a near peak at 24 h without any addition. Based on the above results it can be postulated that treatment of rice bran by *Candida utilis* may improve feed quality by reducing the fat content, increasing the crude protein content and adding the function of yeast for stimulating rumen microbes.

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