Effects of Strains of *Saccharomyces cerevisiae* and Incubation Conditions on the *In vitro* Degradability of Yeast and Roughage

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ABSTRACT : The *in vitro* degradability of yeast and the effect of yeast on the *in vitro* degradability of forage may differ in terms of the specific yeast strains or their incubation conditions. Thus in experiment 1, two strains of sake yeast (strainK7 and strainK9) and one strain of bakers' yeast (KY5649) were incubated in an aerobic condition. In experiment 2, aerobically or anaero bically incubated K7 was used for investigating the *in vitro* degradability of yeast, the effect of yeast on the *in vitro* degradability of forage, and the degradability of yeast by pepsin and pronase treatment. The *in vitro*degradability of bakers' yeast was significantly ($p<0.05$) higher than those of sake yeasts. The *in vitro* degradability of anaerobically incubated yeast was significantly ($p<0.01$) higher than that of aerobically incubated yeast. The degradability of bakers' yeast by pepsin treatment was significantly ($p<0.01$) higher than that of the sake yeasts. The degradability of bakers' yeast by pronase treatment was slightly higher than that of the two sake yeasts, while the degradability of anaerobically incubated yeast by both enzymes, respectively, was significantly ($p<0.01$) higher than that of aerobically incubated yeast. The degradability of forages was increased significantly ($p<0.05$) by the addition of yeasts. The degradability of roughage by sake yeast tended to be higher than that by the bakers' yeast. The degradability of roughage was significantly ($p<0.05$) higher by anaerobically incubated yeast than by aerobically incubated yeast. Given the above results, it seems that *in vitro* degradability of yeast and the magnitude of the increment of roughage degradation differ among the yeast strains and their incubation conditions. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 354-357)***

Key Words : Yeast, Degradability, Rice-straw, Italian-ryegrass, Sorghum Incubation-condition

INTRODUCTION

Yoon and Stern (1995) presented a model depicting the action of yeast in ruminants. Using this model, they concluded that the increment of production brought about by yeast supplementation is due to the increased rate of fiber digestion and/or the increased microbial protein synthesis due to the activation of the microbial population, and to the stabilization of the ruminal environment through the utilization of lactic acid and ammonia. Concerning the effects of yeast addition on ruminant production, Piva et al. (1993) reported that milk yields were increased by dietary supplementation with yeast. Fallon and Harte (1987) and Williams et al. (1987) reported that body weight gain increased from yeast supplementation. With regard to the effects of yeast addition on rumen microbes, Wiedmeier et al. (1987), Harrison et al. (1988) and Dawson et al. (1990) reported that fibroratic bacteria increased due to the effects of yeast,and Ando et al. (2004) reported that dry matter digestibility increased by dried beer yeast addition, and also Nisbet and Martin (1991) reported that the number of lactic acid-utilizing bacteria were increased by yeast. Furthermore, Dawson and Hopkins (1991) and Erasmus et al. (1992) reported that different strains of yeast affect different microbes to different degrees. The reason for this phenomenon was not discussed. It can be postulated that differences in the effect of yeast upon ruminal microbes or ruminant performance are related to the differences in the yeasts' metabolic functions or cell wall structures. On the other hand different yeasts' ability to synthesize ethanol and their tolerance to ethanol are also related to their metabolic functions or cell wall structures (Hara et al., 1978; Inoue et. al., 2000; Ogawa et al., 2000). Sake yeast is much stronger in ethanol synthesis and shows a greater tolerance to ethanol than does bakers' yeast. Sake yeast generates energy by oxidizing sugars to water and carbon deoxidized in aerobic conditions. On the other hand, it generates energy by fermenting sugars to ethanol and carbon deoxidized in anaerobic conditions. So, there may be difference in yeasts' metabolic functions or cell wall structures between sake yeast and bakers' yeast, and between aerobically incubated sake yeast and anaerobically incubated one. Therefore, it can be postulated that there may be differences in the effects of these yeasts upon ruminal microbes or ruminant performance between sake yeast and bakers' yeast and between aerobically incubated sake yeast and anaerobically incubated types. In the present study, the differences in the

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Table 1. Chemical composition of roughages

	Roughages		
	Sorgum	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

Table 2. Effect of yeast strain on the *in vitro* and enzymic degradability

Item	Bakers' yeast	K-7	K-9
In vitro degradability	77.8 ± 4.15^{a}	70.3 ± 2.29^{b}	72.2 ± 4.02^{b}
Pepsin degradability	75.8 ± 0.41^{A}	71.6 ± 0.45^{B}	72.8 ± 0.51^{B}
Pronase degradability	82.9 ± 1.60	81.9 ± 2.23	81.8 ± 0.51

[a, b] Significant in different letters ($p<0.05$).
[A, B] Significant in different letters ($p<0.01$).

effect of sake yeast and bakers' yeast (experiment 1), and in the effect of aerobically incubated sake yeast and anaerobicaly incubated one (experiment 2) on the *in vitro* degradability of sorghum, rice straw and Italian ryegrass were investigated. The *in vitro* degradability of the yeasts was also investigated. Finally, the response of yeasts to enzymic treatment using pepsin and pronase was tested in order to investigate the relationship between the *in vitro* degradability of yeast or the effect of yeasts on *in vitro* roughage degradability and cell wall contents.

MATERIALS AND METHODS

Yeasts and their incubation

Two strains of sake yeast (Strain K7 and Strain K9) and bakers' yeast (KY5649) were used in experiment 1. One loopful of yeast grown on YM broth-solid plate media was placed into 400 ml YM-broth solution media. These solutions were incubated for 48 h at 24°C in aerobic conditions. After incubation, the yeast was collected by centrifuge (500 G, 5 minutes). Centrifuged yeasts equivalent to DM 0.1 g were put into 50 ml centrifuge tubes, then were dried at 60°C for 48 h.

Sake yeast, Strain K7 was used in experiment 2. The yeast was incubated for 48 h at 24°C in both aerobic and anaerobic conditions. After incubation, the yeast was treated in the same manner as that in experiment 1.

In vitro degradability

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 chemical composition of roughages was shown in Table 1. Measurements of *in vitro* degradability were carried out three times with two replications for each treatment.

Table 3. Effect of incubation condition on the *invitro* and enzymic degradability

Item	Aerobic	Anaerobic
In vitro degradability	81.4 ± 3.54^{A}	89.0 ± 2.29^{B}
Pepsin degradability	71.1 ± 0.84^{A}	74.4 ± 0.30^{B}
Pronase degradability	76.8 ± 0.47^{A}	80.9 ± 0.48^{B}

[A, B] Significant in different letters ($p<0.01$).

Enzyme treatment

For the enzymic treatments, centrifuged yeast equivalent to DM 0.2 g was placed into 50 ml centrifuge tubes. The degradability of each yeast by pepsin was measured following the method of Tilly and Terry (1963), while the degradability of each yeast by pronase was measured following the method of Abe et al. (1979). Six replications were carried out for each enzymic treatment.

Statistical method

Duneet's multiple comparison procedure (Dunett, 1955) was used as statistical analysis method.

RESULTS

Effect of yeast strains on the *in vitro* degradability and enzymic degradability of yeasts

Table 2 shows the effects of yeast strains on the *in vitro* and enzymic degradability of the yeasts. The *in vitro* degradability of all three were above 70%, with the degradability of bakers' yeast significantly ($p<0.05$) higher than that of the two sake yeasts. The degradability of K9 was slightly higher than K7. The degradability of bakers' yeast was significantly ($p<0.01$) higher than those of two sake yeasts by pepsin treatment. Between the two sake yeasts, the degradability of K9 was slightly higher than that of K7. The degradability of bakers' yeast was slightly higher than that of the two sake yeasts by pronase treatment. No difference in pronase response was seen between the two sake yeasts.

Effect of incubation condition on the *in vitro* degradability and enzymic degradability of yeasts

Table 3 shows the effects of yeast strains on the *in vitro* and enzymic degradability of the yeasts. The *in vitro* degradability of the yeast under both incubation conditions was above 80%, with the degradability of anaerobicaly incubated yeast significantly ($p<0.01$) higher than that of the aerobically incubated one. The degradability of anaerobically incubated yeast was significantly ($p<0.01$) higher than that of aerobically incubated ones by both enzyme treatments.

Effect of yeast strains on roughage degradability

In Table 4, the 24 h degradability of the three roughage types and the effects of different yeasts strains upon this

Table 4. Effects of yeast strains on the roughage degradation

	Roughages			
	Sorgum	Italian-ryegrass	Rice-straw	Average
No addition	41.9±0.58	42.8±0.35	27.1±0.41	37.3[a]
Bakers' yeast	48.4±1.03	52.2±0.99	30.4±0.56	43.7[b]
K7	48.8±0.81	54.1±0.43	33.0±0.75	45.3[b]
K9	50.3±1.25	52.8±0.72	30.7±0.97	44.6[b]

[a,b] Significant in different letters ($p<0.05$).

Table 5. Effects of incubation condition on the roughage degradation

	Roughages			
	Sorgum	Italian-ryegrass	Rice-straw	Average
No addition	35.2±1.03	36.4±0.86	20.3±0.93	30.6[a]
Aerobic	47.4±0.92	45.0±1.10	24.7±0.87	39.0[b]
Anaerobic	40.4±1.07	39.8±0.98	23.1±0.91	34.4[c]

[a,b,c] Significant in different letters ($p<0.05$).

figure are shown. The addition of the yeasts increased the degradability of the forages significantly ($p<0.05$). The degradability of roughage tended to be higher for sake yeast supplementation than for the addition of bakers' yeast.

Effect of incubation conditions on the roughage degradability

In Table 5, the 24 h degradability of three roughage types and the effects of incubation conditions upon this figure. The addition of the yeasts increased the degradability of the forages significantly ($p<0.05$). The degradability of roughage was significantly ($p<0.05$) higher for aerobically incubated yeast than for the addition of anaerobically incubated yeast.

DISCUSSION

The cell wall of yeast consists of β-glucan, mannan, chitin, and protein (Cid et al., 1995). All cell wall proteins were removed by pronase treatment (Abe et al., 1979); hence, the undegradable portion after pronase treatment contains β-glucan, mannan, and chitin. In contrast, pepsin treatment could not remove all of the cell wall protein (Moir 1972; Morrison 1973). By the pronase treatment, the degradability of bakers' yeast was slightly, but was not significantly higher than that of the two sake yeasts, while the degradability of anaerobically incubated yeast was significantly higher than that of the aerobically incubated one.By the pepsin treatment, the degradability of bakers' yeast was significantly higher than it did the two sake yeasts, and the degradability of anaerobically incubated yeast was significantly higher than that of the aerobically incubated one. These results show that the cell wall structure of sake yeasts is firmer than that of bakers' yeast, and that the cell wall structure of aerobically incubated yeast is firmer than that of the anaerobically incubated one. Given the above results, the differences in the yeasts' cell walls may be responsible for the higher *in vitro* degradability of bakers' yeast than sake yeast and for the higher *in vitro* degradability of anaerobically incubated yeast than that of the aerobically incubated one.

Wiedmeier et al. (1987), Harrison et al. (1988) and Dawson et al. (1990) reported that the addition of yeast increased fiber degradation. In the present study, the addition of yeast increased the degradability of forages. And our results supported those of the prior reports. The increment of forage degradability tended to be higher in the sake yeasts than in the bakers' yeast and was significantly higher in aerobically incubated yeast than in the anaerobically incubated one. These results suggest that there are differences between different yeasts' effects upon ruminal microbes, thus supporting the reports of Dawson and Hopkins (1991) and Erasmus et al. (1992). It was also pointed out that the reason for the above differences might be due to the differences in the yeasts' nutrient contents or in the yeasts' cell wall structures.

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