Manipulation of Rumen Fermentation by Yeast: The Effects of Dried Beer Yeast on the *In vitro* Degradability of Forages and Methane Production

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ABSTRACT : The effects of the addition of yeast on *in vitro* roughage degradability and methane production were investigated in order to clarify the effects of yeast on the rumen microbes and to establish methods of rumen manipulation. Three roughages (whole crop corn, rice straw and Italian ryegrass) were incubated for 3, 6, 12 and 24 h with or without dried beer yeast following the method described by Tilley and Terry. Using the same method, these roughages were incubated with or without yeast extract, albumin or purified DNA. *In vitro* methane production was measured with or without dried beer yeast at 12 and 24 h. The degradability of yeast was found to be 57 and 80% at 12 and 24 h, respectively. The rate of degradation of fraction b was 6.16%/h. There was a significant increase in roughage degradability at 6 h ($p<0.05$), 12 h ($p<0.05$) and 24 h ($p<0.01$) by dried yeast addition. The degradability of all three roughages was higher in the samples treated with yeast extract than in the no addition samples except in the case of rice straw incubated for 12 h. Nevertheless, the magnitude of increment was smaller with the addition of yeast extract than without the addition of yeast. With the addition of purified DNA, there were significant increases in roughage degradability at 6 h ($p<0.01$), 12 h ($p<0.01$) and 24 h ($p<0.05$); however, higher degradability values were detected in the samples to which albumin was added, particularly at 6 h. If the degradability values of the no addition samples with those of samples containing yeast, yeast extract, DNA and albumin were compared, the largest difference was found in the samples to which yeast was added, although it is worth noting that higher values were observed in the yeast extract samples than in the DNA or albumin samples, with the exception of the case of rice straw incubated for 24 h. Methane production was significantly increased at both 12 and 24 h incubation. The increment of roughage degradation and methane production brought about by the addition of dried beer yeast to the samples was thought to be due to the activation of rumen microbes. Water soluble fraction of yeast also seemed to play a role in ruminal microbe activation. The increment of degradability is thought to be partially due to the addition of crude protein or nucleic acid but it is expected that other factors play a greater role. And those factors may responsible for the different effects of individual yeast on ruminal microbes. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 1 : 68-72)***

Key Words : Yeast, Ruminal Microbes, Whole Crop Corn, Rice Straw, Italian-ryegrass, Methane

INTRODUCTION

Many methods of rumen manipulation for increasing the productivity of animals and for improving the quality of meat or milk have been proposed (Chalupa, 1977). In applying these methods, it is necessary to take into consideration their effects on the host animal, on the products in question, and on the environment; the durability of these effects is also worthy of attention. If rumen manipulation is achieved through natural substances, such as those used in feed, no problems should occur. Yeast, for example, is widely used in foods, medicines and feeds. It has been reported that feeding yeast to dairy or beef cattle increases feed intake (Williams et al., 1991), milk production (Williams et al., 1991; Piva et al., 1993) and body weight gain (Fallon and Harte, 1987; Williams et al., 1987). Additionally, Yoon and Stern (1995) present a model depicting the action of fungal cultures in ruminants. Using this model, they concluded that the increment of production response brought about by yeast supplementation is due to an increased rate of fiber digestion and/or increased microbial protein synthesis following an increase in the microbial population. More information about the effects of yeast upon ruminal microbes, and the magnitude of these effects is needed for establishing the methods of rumen manipulation using the yeast. Thus, the present study examines the *in vitro* degradability of Whole crop corn, Rice straw and Italian ryegrass incubated with or without dried beer yeast or its extract in order to clarify the effect of yeast on the activation of ruminal microbes. In order to distinguish the effects on ruminal microbes of the addition of yeast from the potential supplemental effects of crude protein or nucleic acid, the *in vitro* roughage degradability was also investigated in samples to which albumin or purified DNA had been added. Furthermore, *in vitro* methane production was measured to clarify the effects of dried beer yeast on the activity of rumen microbes.

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Table 1. Degradation characteristics of dried beer yeast

Dry matter degradability (%)				a (%)	b (%)	c (%/h)
3 h	6 h	12 h	24 h			
32.6	33.1	56.8	80.3	11.5	88.6	6.2

a: Easily degradable fraction. b: Difficultly degradable fraction.
c: Degradation rate of b.

MATERIALS AND METHODS

Measurement of forage degradability with dried beer yeast

Whole crop corn, Rice straw and Italian ryegrass which were milled and screened through a 2 mm mesh were used as roughage. Control and samples under 7 different treatment conditions were prepared: Whole crop corn, Rice straw, Italian ryegrass, yeast (dried beer yeast, commercially purchased, produced in beer industry), Whole crop corn+yeast, Rice straw+dried beer yeast (commercially purchased, produced in beer industry), and Italian ryegrass+yeast. The amount of yeast and forage were 0.2 g. And they were put into a 50 ml centrifuge tube. Rumen fluid was collected from two ruminally cannulated Japanese black cows fed Italian ryegrass hay *ad libitum*. (About 6 kg/day). Incubation and measurement of dry matter degradability were carried out for 3, 6, 12 and 24 h following the method described by Tilley and Terry (1963). But pepsin digestion was not carried out without pepsin digestion. The degradability of the roughages was calculated by total dry matter degradability and that of dried beer yeast. The degradabilities of the roughages and the yeast were correlated to the incubation time using the equation formula proposed by Orskov and McDonald (1979).

Measurement of forage degradability with beer yeast extract

Yeast extract solution was produced by adding 1,000 ml water to 10 g dried beer yeast and storing at 4°C for 16 h. 20 ml of extract solution (equivalent to 0.2 g dried yeast) was placed in 50 ml centrifuge tubes and the samples were dried at 60°C for 48 h. The in vitro degradability of Whole crop corn, Rice straw and Italian ryegrass at incubation times of 3, 6, 12 and 24 h was measured following the method mentioned above with or without the dried yeast extract. The degradability of roughages was correlated to the incubation time by the equation formula used above.

Measurement of forage degradability with DNA or albumin

The *in vitro* degradability of Whole crop corn, Rice straw and Italian ryegrass at incubation times of 3, 6, 12 and 24 h was measured with or without albumin or purified DNA. The amount of albumin added was 0.0994 g, which gives a CP content equivalent to that of 0.2 g dried beer yeast; likewise, the amount of DNA added was 0.006 g, which gives a nucleic acid content equivalent to that of 0.2 g dried beer yeast.

Measurement of *in vitro* methane production

In vitro methane production was measured using the method described by Gamo et al. (2001). 5 g of Orchardgrass hay was used as substrate and 1 g of dried beer yeast was added. Incubation was performed in 2 times on the samples with yeast and in 3 times on the control samples. Incubations were carried out for 24 h. Cumulative values for 12 and 24 h were measured.

RESULTS

Table 1 shows the degradability characteristics of the digestion of yeast. At both 3 and 6 h incubation time, degradability was about 33%, while degradabilities of 57% and 80% were found at 12 and 24 h, respectively. The fraction of a was 11.5%; the fraction of b was 88.6% and the degradation rate of b was 6.16%/h.

The degradability characteristics of the digestion of roughages with or without yeast are shown in Table 2. At 3 h incubation time, the addition of yeast had no effect on roughage degradability, however there was a significant increase in roughage degradability at 6 h ($p<0.05$), 12 h ($p<0.05$) and 24 h ($p<0.01$). The addition of yeast had no effect on the percentage of fraction a or b. The degradation rate of fraction b was significantly higher ($p<0.01$) in the

Table 2. Effect of dried beer yeast on the degradability of roughages

Roughage	Treatment	Dry matter degradability (%)				a (%)	b (%)	c (%/h)
		3 h	6 h	12 h	24 h			
Corn	No. addition	18.4	19.5	31.1	39.3	14.4	85.6	1.5
	Yeast	15.6	20.1	37.5	50.6	9.4	90.6	2.6
Rice straw	No. addition	13.0	10.5	12.7	16.9	10.5	89.5	0.3
	Yeast	12.7	13.9	22.8	27.9	10.5	89.5	1.0
Italian-ryegrass	No. addition	19.7	21.2	26.9	27.8	19.5	80.5	0.5
	Yeast	21.4	24.5	29.0	38.3	18.8	81.2	0.5
Average	No. addition	17.0	17.0	23.6	28.0	14.8	85.2	0.8
	Yeast	16.6	19.5*	29.8*	38.9**	12.9	87.1	1.6**

a: Easily degradable fraction. b: Difficultly degradable fraction. c: Degradation rate of b.
Significant differences between control samples and yeast samples: * $p<0.05$, ** $p<0.01$.

Table 3. Effect of dried beer yeast extract on the degradability of roughages

Roughage	Treatment	Dry matter degradability (%)				a (%)	b (%)	c (%/h)
		3 h	6 h	12 h	24 h			
Corn	No. addition	16.3	19.7	35.6	43.0	12.9	87.1	1.9
	Yeast	16.6	22.6	44.3	54.1	11.6	88.4	2.9
Rice straw	No. addition	7.4	9.0	13.8	21.0	5.0	95.0	0.1
	Yeast	9.0	10.0	12.7	22.4	5.8	94.2	0.1
Italian-ryegrass	No. addition	16.1	24.4	26.7	40.9	13.8	86.2	1.6
	Yeast	22.8	25.3	29.5	45.5	17.2	82.8	1.7
Average	No. addition	13.3	17.7	25.4	35.0	10.6	89.4	1.2
	Yeast	16.1	19.3*	28.8	40.7	11.5	88.5	1.6

a: Easily degradable fraction. b: Difficultly degradable fraction. c: Degradation rate of b.
Significant differences between no addition sample and yeast extract added samples: * $p<0.05$. ** $p<0.01$.

Table 4. Effect of DNA on the degradability of roughages

Roughage	Treatment	Dry matter degradability (%)				a (%)	b (%)	c (%/h)
		3 h	6 h	12 h	24 h			
Corn	No. addition	16.3	16.5	32.5	56.7	2.5	97.5	3.3
	DNA	13.4	24.6	40.7	61.5	4.5	95.5	3.8
Rice straw	No. addition	5.8	10.3	10.4	23.7	2.9	97.1	1.0
	DNA	11.9	14.1	16.0	27.1	8.4	91.3	0.9
Italian-ryegrass	No. addition	10.3	19.2	25.4	47.3	3.6	96.4	2.5
	DNA	19.1	26.6	30.5	48.0	14.5	85.5	2.0
Average	No. addition	10.8	15.3	22.8	42.6	3.0	97.0	2.2
	DNA	14.8	21.8**	29.1**	45.5*	9.1*	91.0*	2.2

a: Easily degradable fraction. b: Difficultly degradable fraction. c: Degradation rate of b.
Significant differences between no addition samples and DNA added samples: * $p<0.05$, ** $p<0.01$.

Table 5. Effect of albumin on the degradability of roughages

Roughage	Treatment	Dry matter degradability (%)				a (%)	b (%)	c (%/h)
		3 h	6 h	12 h	24 h			
Corn	No. addition	16.3	16.5	32.5	56.7	2.5	97.5	3.3
	Albumin	16.6	21.5	45.8	59.3	8.2	91.8	3.5
Rice straw	No. addition	5.8	10.3	10.4	23.7	2.9	97.1	1.0
	Albumin	11.7	14.6	17.3	29.2	8.4	91.3	1.0
Italian-ryegrass	No. addition	10.3	19.2	25.4	48.3	3.6	96.4	2.5
	Albumin	18.6	25.2	26.3	48.3	12.2	87.8	2.1
Average	No. addition	10.8	15.3	22.8	42.6	3.0	97.0	2.2
	Albumin	15.6	20.4**	29.8	45.6	9.6**	90.4**	2.2

a: Easily degradable fraction. b: Difficultly degradable fraction. c: Degradation rate of b.
Significant differences between no addition samples and albumin added samples: * $p<0.05$, ** $p<0.01$.

yeast addition than in no yeast addition.

Table 3 shows the degradability characteristics of digestion of roughages with or without yeast extract. In Italian ryegrass and Whole crop corn, higher degradability values were observed for the samples incubated with the yeast extract; this was also true of the Rice straw samples except at 12 h incubation. The degradation rate of fraction b was higher with the addition of yeast extract except in the case of rice straw. The differences between the no addition samples and the test samples were larger with the addition of yeast than with the addition of yeast extract.

The degradability characteristics of digestion of roughages with or without DNA are shown in Table 4. At 3 h incubation time, the addition of DNA had no effect on roughage degradability, however there were significant increases in roughage degradability at 6 h ($p<0.01$), 12 h ($p<0.01$) and 24 h ($p<0.05$). The percentage of fraction a was increased ($p<0.05$) and the percentage of fraction b was significantly decreased ($p<0.05$) by the addition of DNA.

Table 5 shows the degradability characteristics of digestion of roughages with or without albumin. Consistently higher degradability values were detected with the addition of albumin than with the addition of DNA, particularly at 6 h incubation time, when a significant difference ($p<0.01$) was observed. The percentage of fraction a was increased and the percentage of fraction b was significantly decreased ($p<0.05$) by the addition of albumin.

Table 6 shows the differences in degradability between the no addition and test samples. The difference was largest in the yeast samples at 24 h incubation time. The yeast extract samples showed generally higher values than the

Table 6. Difference between no addition and added samples

Roughage	Treatment	Difference of dry matter degradability (%)			
		3 h	6 h	12 h	24 h
Corn	Yeast	-2.7	0.6	6.4	11.2
	Extract	0.3	2.9	8.7	11.1
	DNA	-2.9	8.1	8.2	4.8
	Albumin	0.3	5.0	13.3	2.6
Rice straw	Yeast	-0.3	3.4	10.1	11.0
	Extract	1.6	1.0	-1.1	1.4
	DNA	6.1	3.8	5.6	3.4
	Albumin	5.9	4.3	6.9	5.5
Italian- ryegrass	Yeast	1.7	3.3	2.1	10.5
	Extract	4.6	2.8	0.9	6.7
	DNA	8.8	7.4	5.1	0.7
	Albumin	8.3	6.0	0.9	1.0
Average	Yeast	-0.4^{a}	2.4^{a}	6.2	10.9^{a}
	Extract	2.2^{ab}	2.2^{a}	2.8	6.4^{a}
	DNA	4.0^{b}	6.4^{b}	6.3	3.0^{b}
	Albumin	4.8^{b}	5.1^{b}	6.4	3.0^{b}

Significant differences between different letters ($p<0.05$).

Table 7. *In vitro* methane production (ml)

Incubation time	Treatment	
	Control	Beer yeast
12 h	14.1	44.1**
24 h	28.4	60.2**

** Significant difference between control and yeast samples ($p<0.01$).

DNA or albumin samples except in the case of Rice straw. Nevertheless, all values obtained for the yeast extract samples were lower than that of those obtained for the yeast samples. At 12 h incubation, the differences in degradability in the test samples varied according to the type of roughage. At 3 h and 6 h incubation, higher values were observed in the DNA and albumin samples than in the yeast samples, except for the case of Whole crop corn+DNA at 3 h incubation. The effect of the addition of yeast on *in vitro* methane production is shown in Table 7. The amount of methane produced at 12 h was 14.1 ml for the control samples and 44.1 ml for the yeast samples; that is, the addition of yeast significantly increased methane production ($p<0.01$). A significant difference ($p<0.01$) was also observed in the methane production for 24 h,which was calculated at 28.4 ml for the control samples and 60.2 ml for the dried beer yeast samples.

DISCUSSION

Ruminal microbes play an important role in forage degradation. Specifically, the numbers of rumen microbes and/or their activity contribute to the magnitude of forage degradation (Hungate, 1966). Increased concentrations of ruminal fibrolytic bacteria have been observed to result from yeast supplementation (Wiedmeier et al., 1987; Harrison et al., 1988; Dawson et al., 1991). In the present study, forage degradation was increased by the addition of dried beer yeast. Thus, as in agreement with the above reports, it can be concluded that the addition of yeast activates rumen microbes.

In the present study, heat dried yeast was used, yet addition of this yeast activated rumen microbes, providing additional evidence in favor of the conclusion presented by Dawson et al. (1990) that heat-killed yeast remains able to stimulate rumen microbes. It was also found in the present study that the addition of yeast extract increases forage degradability. Nisbet and Martin (1991) reported that yeast extract stimulates rumen microbes, therefore, water soluble fraction of yeast seems to play a role in ruminal microbe activation. Yeast, however, is rich in protein and nucleric acid, and the effects of yeast on rumen microbe activation might be due to some supplemental effect of these substances. The results show that at in 24 h incubation, the increment of degradability was larger in yeast samples than in albumin or DNA samples, strongly suggesting that the increment of degradability is only partially due to crude protein or nucleic acid and that it is likely that other factors are primarily responsible for the increment of degradability. At 3 h and 6 h incubation, consistently higher degradability values were observed in DNA and albumin samples than in yeast samples, except in the case of the whole crop corn+DNA samples at 3 h incubation. This is explained by the fact that albumin and DNA exist in a dissolved form in buffer solution, but yeast is not entirely degradated during the early stages of incubation. There is close relationship between the digestibility of roughage and methane production (Johnson and Johnson, 1995).

In the present study, both roughage degradability and methane production were increased by the addition of yeast, indicating that yeast increases the activity of rumen

microbes. The results of present study suggest that yeast activates rumen microbes not only through the supplemental effects of crude protein or DNA, but also by other means. It has been reported that the effects of yeast on ruminal microbes vary according to the specific strain of yeast (Dawson and Hopkins, 1991; Newbold et al., 1995). Thus, it can be postulated that some factor other than crude protein or DNA may be responsible for the different effects of individual yeasts on ruminal microbes.

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