

The Effect of Use of Tofu Cake, Corn Distiller's Dried Grains  
with Solubles and Enzyme on Fermentation Quality of Fermented  
Total Mixed Ration  
with Second Cutting Reed Canarygrass (*Phalaris arundinacea* L.)

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# The Effect of Use of Tofu Cake, Corn Distiller's Dried Grains with Solubles and Enzyme on Fermentation Quality of Fermented Total Mixed Ration with Second Cutting Reed Canarygrass (*Phalaris arundinacea* L.)

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## Summary

This experiment was carried out to examine the effect of use of tofu cake, two kinds of corn distiller's dried grains with solubles which were made from different manufacturers and enzyme additive on the fermentation quality of total mixed ration based on second cutting reed canarygrass (RCG). Two RCG mixed ratios were set 45% and 65% in fresh matter basis and enzyme (the trade name is "Procelase10<sup>®</sup>") was added at 0.2% level by the method of pouch. In fermentation quality, pH was higher and lactic acid content was lower at second cutting RCG than at first cutting RCG in previous report. The V-score and Flieg's score, however, were high because almost none of the butyric acid was detected. In addition, in both RCG mixed ratios, the improvement effect of fermentation quality was weak by enzyme addition. It was thought that moisture content was lower at second cutting RCG than at first cutting RCG as the cause of high evaluation of fermentation quality in second cutting RCG.

**Key words** : fermentation quality; fermented total mixed ration; second cutting reed canarygrass

## Abbreviations

ADF, acid detergent fiber; ADL, acid detergent lignin; DDGS, corn distiller's dried grains with solubles; DM, dry matter; FM, fresh matter; IVDMD, *in vitro* dry matter digestibility; NDF, neutral detergent fiber; RCG, reed canarygrass; TC, tofu cake; TMR, total mixed ration; VBN, volatile basic nitrogen; WSC, water soluble carbohydrate

## Introduction

Dry matter intake and milk production are greater when assorted feed is given than when only grazing management is performed. Further increases in dry matter intake and milk production can be obtained when a total mixed ration (TMR) is given (Bargo *et al.* 2002). The use of TMR also requires a smaller workforce due to mechanization. Therefore, methods for preparing

TMR are widespread (Keane *et al.* 2006). Fermented TMR was developed to make up for deficiency, such as aerobic deterioration, of preservation of fresh TMR (Shioya *et al.* 2007). Many studies on fermented TMR have been reported (Sudo *et al.* 2007; Pinos-Rodríguez *et al.* 2008; Yokoyama *et al.* 2009a, b; Cao *et al.* 2009b).

As the result of investigation on re-growth characteristics of reed canarygrass (*Phalaris arundinacea* L., RCG) grassland after harvesting, it was cleared that

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the yield was higher for RCG than for orchardgrass (*Dactylis glomerata* L.) (Hoshino 1983). The intake for the first and second cutting RCGs were lower than for the first cutting timothy (*Phleum pratense* L.) (Iuchi *et al.* 2008). The *in vitro* dry matter digestibility (IVDMD) was lower for the second cutting RCG than for the first cutting RCG (Otake *et al.* 1993). The high yields of the first and second cutting RCGs emphasize the importance of determining the most effective use of the second cutting.

Although the fibrolytic enzymes were capable of increasing IVDMD, the enzyme mixture that was most efficacious *in vitro* did not affect the lactational performance of dairy cows (Elwakeel *et al.* 2007). Addition of fibrolytic enzymes for TMR can be used to improve milk production in lactating cows. (Kung *et al.* 2002). The fermentation quality of the first cutting RCG silage and RCG TMR silage was reported to be improved by adding the enzyme (Tagawa *et al.* 2001; 2010; 2011). However, no study has yet investigated the effect of enzyme treatment on the fermentation quality of fermented TMR based on second cutting RCG.

In this study, the effective use of the second cutting RCG for making fermented TMR was examined the effects of tofu cake (TC), two types of corn distiller's dried grains with solubles (DDGS) produced in different ethanol production factories and enzyme comparing with previous report (Tagawa *et al.* 2011), which was an experiment used first cutting RCG.

## Materials and Methods

### TMR preparation

TMR was prepared using RCG, TC, DDGS (DDGS-A and -B), beet pulp pellet, corn, soybean meal, calcium carbonate, and the enzyme as raw materials. Still, since we were not allowed to disclose the names of actual factory sources, the two types of DDGS were designated as DDGS-A and -B in this paper. Second cutting RCG was obtained at 19th July 2009 from field science center, faculty of agriculture, Yamagata university, Japan. The second cutting was harvested at the heading stage (the average plant length of 94 cm) and the harvested RCG

was immediately cut into 2 cm pieces. Mixing ratio of RCG at 45% and 65% of fresh matter (FM), were determined to be sufficient for nutritive values (total digestible nutrients and crude protein contents) and to be the maximum RCG ratio as much as possible in Japanese feeding standard for dairy cattle. The second cutting was harvested 60 days after the first cutting, which this was the same as the average period between the first and second cutting in Hokkaido (Iuchi 2008). Fertilization (kg/10a) for grassland was done nitrogen: 8.0, phosphate: 4.0 and potassium 4.0 on 24th March, and nitrogen: 5.3, phosphate: 2.7 and potassium: 2.7 on 12th June and 24th August by compound fertilizer in 2009. The blending ratio of RCG-based fermented TMR was the same (45% and 65%) in FM basis as that described in a previous report (Tagawa *et al.* 2011) and 0.2%FM of the enzyme (the trade name; "Procelase10<sup>®</sup>", Meiji Seika Pharma Co., Ltd., Tokyo) was added. "Procelase10<sup>®</sup>" contained 2,000 IU/g of cellulase and 10,000 IU/g of protease. The pouch method (Tanaka and Ohmomo 1995) was adopted for fermented TMR, and the method was repeated three times. The prepared fermented TMR was stored at room temperature.

### Chemical analysis

After one month of storage, the fermented TMR was unsealed from pouch. Fermentation products of the TMR silages were determined from cold water extracts. The extract was analyzed for pH value and content of lactic acid, volatile fatty acid (VFA), volatile basic nitrogen (VBN), and total nitrogen. The analytical methods used in this study were the same as those described in a previous report (Tagawa *et al.* 2011). The analytical results were used to calculate the Flieg's score and V-score (Society of use of self-supplied feed 2009).

### Statistical analysis

All data were evaluated by using two-way analysis of variance of the General Linear Model procedure in SAS (1990) i.e.; one factor was different materials which were TC, DDGS-A and -B, and another factor was with or without additive enzyme. When a significant difference was observed at the 5% level, Duncan's multiple range test was performed.

## Results and Discussion

The second cutting of RCG used for fermented TMR had the following characteristics: moisture content; 70.3%, crude protein content in the dry matter (DM) basis; 16.5%, neutral detergent fiber (NDF) ; 65.1%DM, acid detergent fiber (ADF); 38.2%DM, acid detergent lignin (ADL); 4.3%DM and water soluble carbohydrate (WSC); 1.2%DM. Compared to the first cutting of RCG harvested in the same field (Tagawa *et al.* 2011), higher values for contents of crude protein, NDF, ADF, and ADL were found in the second cutting RCG. However, the WSC contents were lower (1.2%DM) in the second cutting than in the first cutting (7.2%DM). Corn, beet pulp pellet, TC and DDGS-A used in the present study were the same as those described in the previous report (Tagawa *et al.* 2011). The chemical composition of DDGS varies due to the differences of corn species or of the ethanol production process (Spiehs *et al.* 2002). The corn species differences include the processing form of corn and the use of high-oil contained corn. In addition, the differences in ethanol production include differences in drying time. In this study, the effects of different types of DDGS shipped from different ethanol production factories on the fermentation quality of the fermented TMR have investigated. The present study

used two types of DDGSs imported to Japan. However, differences were not observed in appearance and in analytically determined chemical compositions between DDGS-A and -B. Although it was not clear this reason of no difference, DDGS-A and -B was estimated similar.

Food processing by-products, such as TC (Cao *et al.* 2009a; 2009b), concentrated spirit distillation residue (Yokoyama *et al.* 2009a), rice bran (Cao *et al.* 2009a; 2010), molasses (Cao *et al.* 2010), and green tea grounds (Cao *et al.* 2009b) are reported to improve the nutritive value of TMR. A report described that the use of TC increased the lactic acid content in fermented TMR (Cao *et al.* 2009b). In the present study, the lactic acid content was significantly higher in the enzyme-treated TC group than in the DDGS-A and -B groups at the RCG ratios of 65% and 45% (Table 1 and 2). The moisture contents were below 45% and below 60% when the RCG ratios were 45% and 65%, respectively. The pH value was decreased until 4.3 in the enzyme-treated TC group of first cutting RCG (Tagawa *et al.* 2011). However, pH value decreased to only about 5.0 in this study. There was a significant difference in acetic acid content among all groups, but the difference is small, acetic acid content was between 0.4% and 0.2%. The propionic acid, iso-butyric acid and butyric acid contents were almost not detected in all groups. The Flieg's score and V-score

Table 1. Ingredients and nutritive value of second cutting reed canarygrass TMR<sup>1</sup> silage.

Materials	Evaluated ingredients					
	RCG <sup>2</sup> at 45%			RCG at 65%		
	Tofu cake	DDGS <sup>3</sup> -A	DDGS-B	Tofu cake	DDGS-A	DDGS-B
Ingredients (% of fresh matter)						
Reed canarygrass	45.0	45.0	45.0	65.0	65.0	65.0
Tofu cake	22.5			20.0		
DDGS-A		23.0			23.0	
DDGS-B			23.0			23.0
Beet pulp pellet	5.0	5.0	5.0	5.0	5.0	5.0
Corn	27.5	27.0	27.0	10.0	7.0	7.0
Calcium carbonate	0.6	0.6	0.6	0.6	0.6	0.6
Calculated value of crude protein, neutral detergent fiber and TDN <sup>4</sup> (% of dry matter)						
Crude protein	15.1	16.4	16.4	16.5	15.4	15.4
Neutral detergent fiber	47.2	53.0	53.0	48.3	45.0	45.0
TDN	70.2	68.0	68.0	65.7	72.0	72.0

<sup>1</sup>TMR: total mixed ration. <sup>2</sup>RCG: reed canarygrass. <sup>3</sup>DDGS: corn distiller's dried grains with solubles.

<sup>4</sup>TDN: total digestible nutrients.

Table 2. Fermentation quality of TMR<sup>1</sup> silage containing second cutting reed canarygrass at 45% in fresh weight.

Enzyme <sup>5</sup>	Tofu cake		DDGS <sup>2</sup> -A		DDGS-B		SEM <sup>3</sup>	P <sup>4</sup>		
	- <sup>6</sup>	+ <sup>7</sup>	-	+	-	+		M <sup>8</sup>	E <sup>9</sup>	M×E
Moisture (%)	42.2	44.5	43.4	44.6	42.1	45.4	0.7	NS	NS	NS
pH	5.37	5.03	5.26	5.30	5.25	5.24	0.05	NS	NS	NS
Lactic acid (%FM <sup>10</sup> )	1.32 <sup>b</sup>	1.92 <sup>a</sup>	0.75 <sup>bc</sup>	0.62 <sup>c</sup>	0.71 <sup>bc</sup>	0.59 <sup>c</sup>	0.13	<0.01	NS	NS
Acetic acid (%FM)	0.24 <sup>ab</sup>	0.32 <sup>a</sup>	0.20 <sup>b</sup>	0.20 <sup>b</sup>	0.19 <sup>b</sup>	0.27 <sup>b</sup>	0.01	<0.01	<0.05	NS
Propionic acid (%FM)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS	NS	NS
Iso-butyric acid (%FM)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS	NS	NS
Butyric acid (%FM)	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>ab</sup>	0.01 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00	<0.05	NS	NS
VBN <sup>11</sup> (%T-N <sup>12</sup> )	2.36 <sup>b</sup>	3.71 <sup>a</sup>	2.32 <sup>b</sup>	1.87 <sup>b</sup>	2.10 <sup>b</sup>	2.12 <sup>b</sup>	0.18	<0.05	NS	<0.05
V-score	99.2 <sup>a</sup>	98.7 <sup>b</sup>	99.3 <sup>a</sup>	99.0 <sup>ab</sup>	99.5 <sup>a</sup>	99.4 <sup>a</sup>	0.1	<0.05	<0.05	NS
Flieg's score	99 <sup>a</sup>	99 <sup>a</sup>	97 <sup>ab</sup>	90 <sup>b</sup>	97 <sup>ab</sup>	89 <sup>b</sup>	1	<0.05	NS	NS

<sup>1</sup>TMR: total mixed ration. <sup>2</sup>DDGS: corn distiller's dried grains with solubles. <sup>3</sup>SEM: standard error of the mean. <sup>4</sup>P: probability. <sup>5</sup>M: material. <sup>6</sup>E: mixed of both acid protease and cellulase ("Procelase10<sup>®</sup>", Meiji Seika Pharma Co., Ltd., Tokyo). <sup>7</sup>-: no added anzyme. <sup>8</sup>+: added enzyme. <sup>9</sup>M: material. <sup>10</sup>E: enzyme. <sup>10</sup>FM: fresh matter. <sup>11</sup>VBN: volatile basic nitrogen. <sup>12</sup>T-N: total nitrogen. <sup>a,b,c</sup>: in the same row, no common superscripts are significantly different at  $P < 0.05$ .

Table 3. Fermentation quality of TMR<sup>1</sup> silage containing second cutting reed canarygrass at 65% in fresh weight.

Enzyme <sup>5</sup>	Tofu cake		DDGS <sup>2</sup> -A		DDGS-B		SEM <sup>3</sup>	P <sup>4</sup>		
	- <sup>6</sup>	+ <sup>7</sup>	-	+	-	+		M <sup>8</sup>	E <sup>9</sup>	M×E
Moisture (%)	53.9 <sup>ab</sup>	51.5 <sup>b</sup>	54.2 <sup>ab</sup>	59.6 <sup>a</sup>	57.3 <sup>ab</sup>	55.7 <sup>ab</sup>	1.0	NS	NS	NS
pH	5.08 <sup>a</sup>	4.97 <sup>a</sup>	5.11 <sup>a</sup>	4.95 <sup>a</sup>	5.07 <sup>a</sup>	4.64 <sup>b</sup>	0.05	NS	<0.05	NS
Lactic acid (%FM <sup>10</sup> )	2.16 <sup>ab</sup>	2.40 <sup>a</sup>	1.12 <sup>cd</sup>	1.70 <sup>bc</sup>	1.05 <sup>d</sup>	1.44 <sup>cd</sup>	0.14	<0.01	<0.05	NS
Acetic acid (%FM)	0.35 <sup>ab</sup>	0.38 <sup>a</sup>	0.25 <sup>b</sup>	0.28 <sup>ab</sup>	0.27 <sup>b</sup>	0.32 <sup>ab</sup>	0.01	<0.05	NS	NS
Propionic acid (%FM)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS	NS	NS
Iso-butyric acid (%FM)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS	NS	NS
Butyric acid (%FM)	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.01 <sup>ab</sup>	0.00 <sup>b</sup>	0.01 <sup>a</sup>	0.00 <sup>b</sup>	0.00	NS	NS	NS
VBN <sup>11</sup> (%T-N <sup>12</sup> )	3.69 <sup>ab</sup>	4.07 <sup>a</sup>	2.48 <sup>b</sup>	3.00 <sup>ab</sup>	2.56 <sup>b</sup>	2.56 <sup>b</sup>	0.19	<0.01	NS	NS
V-score	98.3	98.3	98.6	98.8	98.5	98.5	0.1	NS	NS	NS
Flieg's score	100 <sup>a</sup>	100 <sup>a</sup>	98 <sup>b</sup>	100 <sup>a</sup>	97 <sup>c</sup>	98 <sup>bc</sup>	1	<0.01	<0.01	<0.05

<sup>1</sup>TMR: total mixed ration. <sup>2</sup>DDGS: corn distiller's dried grains with solubles. <sup>3</sup>SEM: standard error of the mean. <sup>4</sup>P: probability. <sup>5</sup>M: material. <sup>6</sup>E: mixed of both acid protease and cellulase ("Procelase10<sup>®</sup>", Meiji Seika Pharma Co., Ltd., Tokyo). <sup>7</sup>-: no added anzyme. <sup>8</sup>+: added enzyme. <sup>9</sup>M: material. <sup>10</sup>E: enzyme. <sup>10</sup>FM: fresh matter. <sup>11</sup>VBN: volatile basic nitrogen. <sup>12</sup>T-N: total nitrogen. <sup>a,b,c,d</sup>: in the same row, no common superscripts are significantly different at  $P < 0.05$ .

of the prepared fermented TMR were above 89 and 99, respectively, when the RCG ratio was 45%. These values were above 97 and 98, respectively, when the RCG ratio was 65%. These were considered excellent results for both the Flieg's score and V-score.

Since the WSC content in the RCG used in the present study was only 1.2%DM, and since little WSC was present in TC, DDGS-A and -B, the source of the fermentation substrate with low moisture content led to the excellent lactic acid fermentation was considered to be the same as that described about fermentation quality of fermented TMR by using DDGS (Asada *et al.* 2009).

The desirable moisture content of the fermented TMR was about 50% (Shioya *et al.* 2007; Sudo *et al.* 2007). The results obtained in the present study indicated that undesirable fermentation products such as propionic acid and butyric acid might be controlled with moisture content of less 60%, although moisture contents of above 60% were not investigated in the present study.

The ratio of VBN to total nitrogen was significantly higher in the enzyme-treated TC group (3.71%) than in the untreated TC, DDGS-A and -B groups at the RCG ratio of 45%. At the RCG ratio of 65%, the ratio of VBN to total nitrogen tended to be high in the enzyme-

treated TC group (4.07%). One possible explanation is decomposition of protein caused by the acid protease contained in the enzyme used in this study.

Both DDGS-A and -B used in this study belonged to the yellow golden type, and no large difference in odor was observed between them. Both were made in USA, and these characteristics were similar to each other. The pH value was significantly lower (4.64) in the enzyme-treated DDGS-B group than in other groups at the RCG ratio of 65% ( $p < 0.05$ , Table 3) and lactic acid content in the enzyme-treated DDGS-A and -B groups were 1.7% and 1.4%, respectively ( $p < 0.05$ , Table 3). However, these differences were small and the difference of between DDGS-A and -B was not clear.

VBN value, V-score and Flieg's score were significantly higher in the enzyme-treated DDGS-A group (100) than in the other DDGS groups. The Flieg's score of all the other enzyme-treated or none enzyme-treated DDGS groups were, however, 89 or higher, and these were evaluated as "excellent."

The results obtained in this study revealed that the fermentation quality of second cutting RCG-based fermented TMR by using TC and DDGS was excellent such as V-score and Flieg's score at more 98 or 89, respectively. The reason why moisture content of RCG was lower in second cutting than in first cutting (Tagawa *et al.* 2011), undesirable fermentation such as propionic acid and butyric acid was controlled in spite of high contents of NDF and ADF. Large difference was not seemed to in fermentation quality of fermented TMR using DDGS in which the production company differs. The effects of enzyme addition were significant at acetic acid content and V-score (Table 2), and pH value, lactic acid contents and Flieg's score (Table 3) in 45 and 65% of RCG mixed ratios (Table 2 and 3), respectively. However, it was considered that the effect of enzyme addition on improving of fermentation quality was small; because fermentation quality of fermented TMR of second cutting RCG was good in this study.

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## 2番草リードカナリーグラス発酵TMRの発酵品質に 及ぼす豆腐粕，トウモロコシジスチラーズ グレインソリュブルおよび酵素の影響

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### 摘 要

2番草リードカナリーグラス (*Phalaris arundinacea* L., RCG) を材料とした発酵TMR (Total mixed ration) の発酵品質に及ぼす豆腐粕と製造元の異なる2種類のトウモロコシジスチラーズグレインソリュブルの利用，並びに酵素 (商品名: プロセラゼ10) 0.2%添加効果をRCGの混合割合 (原物) を45%と65%でパウチ法により検討した。先の報告の1番草RCGを供試したときより2番草

RCG発酵TMRの発酵品質のうち，pHは高く乳酸含量は低かった。しかし，酪酸が殆ど検出されなかったためV-スコアとフリーク評点は高かった。また，何れのRCGの混合割合の場合も，酵素添加による発酵品質の改善効果は弱かった。2番草RCGを利用した時の発酵品質の評価が高かった原因に1番草より水分含量が低かったことが考えられた。

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