



Fermentation characteristics, nutrient composition and *in vitro* ruminal degradability of whole crop wheat and wheat straw silage cultivated at dried paddy field

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Abstract

Wheat cropping is a characteristic point of double cropping system following rice cropping in Japan. Feed evaluation of forage wheat is important for dairy production based on the domestic feed resources. The objective of the present study was to determine the preservation and nutritive characteristics of whole crop wheat silage (WCWS) and wheat straw silage (WSS) cultivated in a drained paddy field of local farms. Whole crop wheat harvested at the dough to yellow ripe stages and wheat straw were ensiled in round-bales and stored for 2 to 12 months. Twenty five round-bales of WCWS and 10 round-bales of WSS were investigated in this study. Both WCWS and WSS were made at farmers fields in 4 different sites. The WCWS was well preserved and averaged of pH 4.46, and concentrations of lactate, acetate and butyrate were 19.2, 10.4 and 4.7 g kg⁻¹ on a dry matter (DM) basis, respectively. The mean pH of WSS was 5.46 and it contained lower acids (1.9, 2.2 and 1.0 g kg⁻¹ DM of lactate, acetate and butyrate, respectively), indicating suppressed fermentation due to high DM content. The WCWS had crude protein of 78 g kg⁻¹ DM and neutral detergent fiber (NDF) of 546 g kg⁻¹ DM, and non-fiber carbohydrate (NFC) of 306 g kg⁻¹ DM on average. There was a higher variation in the CP and NFC contents but relatively less variation in the NDF content among the WCWS samples (variance coefficient 22.0, 12.8 and 6.1%, respectively), which as attributed to the difference in the maturity of grain. The WSS had NDF content of 775 g kg⁻¹ DM, mostly cellulose (415 g kg⁻¹ DM). The *in vitro* organic matter degradability (IVOMD) of WCWS and WSS were 56.1 and 29.3%, respectively. The IVOMD of WCWS was not significantly correlated with pH and lactate and acetate content, but slightly correlated with butyrate content and NH₃-N ratio. The higher coefficient of variation for *in vitro* gas production at the initial stages of ruminal incubation of WCWS was associated with the observation of higher coefficient of variation for NFC content as compared to those of fiber fractions such as NDF and ADF. In conclusion, winter cropping of wheat plant at the post-rice cropping can supply fermentable energy in rumen as whole crop silage, and wheat straw also can be useful as fibrous material by ensiling.

Key words: Whole crop wheat silage, wheat straw silage, drained paddy field, *in vitro* rumen degradability.

Introduction

In recent years, forage rice crop production has been developed and established by breeding high-yield cultivars, preservation technology including machinery and ensiling additives, and feeding menu of beef cattle and dairy lactating cattle ¹⁻³. These advancing agricultural technologies can encourage winter forage cropping system with a combination of rice plant in Asian Monsoon regions including Japan, Korea, and southern China. Amongst possible forage crops, the winter wheat plant is one of the highly possible crops as a counterpart of rice plant, because of its relatively higher yield and higher adaptation to agronomic resume conditions such as low temperature and rainfall during the post rice cropping season ^{4,5}.

The whole crop wheat is mainly cultivated on dry upland as reported in Europe, North America and Middle East countries ⁶⁻⁸. In some cases, the crop is also often intercropped with peas or vetches ^{9,10}. The whole crop wheat silage (WCWS) was reported to partially replaced grass silage in the diet for beef and dairy cattle ¹¹. WCWS can also substitute concentrate and grass silage for lactating cows when it was intercropped with peas ¹². The dry matter and nutritional yields of winter wheat crop varies, mainly because of the

differences of the agronomic resume at seeding time after rice crop harvesting which often severely change the growing conditions and this is also associated with soil drainage in the paddy field ^{4,13}.

Therefore, in the present study, the fermentation quality and nutritive characteristics of WCWS and wheat straw silage (WSS) cultivated in paddy field of local farms were examined.

Materials and Methods

Silage preparation and sampling: Wheat crop was grown in well-drained and dried paddy field of local farms after rice cropping at 4 different sites of Mie prefecture, Japan, with a local standard of agronomic resume. Table 1 gives the cultivar, seeding time and harvesting time of the wheat crop.

A total of 25 round-bales of whole crop wheat, harvested at the dough to yellow ripe stages, were made by 8-9 layers wrapping of polyethylene stretch film and stored outside with various preservation periods between 2 and 12 months. Ten round-bales of wheat straw were also made after 1 to 2 days wilting following

grain harvest, and wrapped and stored similar to WCWS. Samples of WCWS and WSS for chemical analysis and *in vitro* digestion test were obtained by 10 spots per bale using a drill sampler (Fujiwara Scientific Company, Japan). Collected spot samples from each bale were thoroughly mixed, and a subsample (around 1 to 2 kg) was used for the various analyses.

Chemical analysis: Dry matter content of the silages was determined by oven-drying at 60°C for 48 hours. The pH and concentrations of organic acids and NH₃-N in the silages were determined with water extracts obtained from 30 g of WCWS and 20 g of WSS which were blended by a homogenizer for 90 s with 210 and 280 ml of distilled water, respectively. The water extracts were filtrated through four layer cheesecloths. The filtrates were subjected pH measurement using a glass electrode pH meter, and stored at -30°C until further analysis. The concentrations of lactic acid and volatile fatty acids (VFA), such as acetate, propionate, and n-butyrate, were analyzed by HPLC equipped with an ion-exclusion column (Shimadzu SCR-102H, 8 mm x 30 mm, Shimadzu Kyoto, Japan) and a conductivity detector. For a preparation of HPLC injection, the extracts was deproteinized by perchloric acid. NH₃-N was determined by a steam distillation method. The V-score was used in evaluating fermentation quality of silage that was computed based on the ratio of VFA on a fresh mater basis and NH₃-N on a total nitrogen (TN) basis. The score is distributed from 0 to 100, low quality silage with higher ratio of NH₃-N and VFA scored lower and vice versa.

Dried samples were ground and allowed to pass through a 1-mm screen prior to chemical analysis. Standard methods¹⁴ were used for determination of organic matter (OM), ether extracts (EE), nitrogen (N) and crude protein (CP). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined using detergent solutions¹⁵. NDF content was analyzed with α -amylase and sodium sulfite before NDF extraction. Hemicellulose and cellulose were calculated from NDF - ADF and ADF - ADL, respectively. Non fiber carbohydrate (NFC) was computed as OM - (CP + NDF + EE).

In vitro ruminal fermentation trial: Samples of WCWS and WSS were incubated in the rumen *in vitro* following the procedure of Uddin *et al.*¹⁶. One g of the sample was weighed into a 120 ml capacity glass vial and incubated anaerobically at 39°C for 72 hours in a 50 ml mixture of rumen fluid and McDougal buffer solution (1:2, v/v). Rumen fluid was collected before the morning feeding from 2 ruminally cannulated Holstein cows fed Italian ryegrass silage. The rumen fluid was then filtered through four layer cheesecloths and mixed with an anaerobic McDougal buffer (1:2, v/v). All procedures of handling rumen fluid were conducted under continuous CO₂ flow into the vials. Glass vials with sample and buffered rumen fluid were covered with rubber cap and aluminium ring and incubated in a shaking (170 rpm) water bath at 39°C. Gas production was recorded using calibrated glass syringe at 3, 6, 9, 12, 24, 48 and 72 h after incubation. At the end of incubation, the residual contents in the vials were collected by centrifugation and stored at -30°C for determination of *in vitro* dry matter (DM) and OM digestibility. The DM and OM of indigested residues were determined and used to calculate *in vitro* DM degradability (IVDMD) and OM degradability (IVOMD). The *in vitro* rumen incubation was replicated twice and their mean value was presented.

Statistical analyses: Among 25 bales of WCWS and 10 bales of WSS, data were expressed as average and maximum and minimum values. Standard deviation and coefficient of variance (%) were also calculated to determine the variation among two types of silages. Correlations between chemical compositions and gas production and degradability parameters were analyzed by PROC CORR of SAS¹⁷.

Results and Discussion

Fermentation characteristics of wheat silage: Samples of the WCWS in the present study showed light brown and golden color, and strong acidic and partially alcoholic smells when they were opened. DM contents of WCWS ranged between 301 and 434 g kg⁻¹ (average of 363 g kg⁻¹), while the larger differences were observed with the WSS ranging from 439 to 732 g kg⁻¹ (average of 637 g kg⁻¹, Table 2). The wide WCWS range was probably due

Table 1. Agronomic characteristics of wheat used in the present study.

Site	Cultivar	Forage type	Date of Seeding	Date of Harvesting	Growth stage at harvest
A	Nishinokaori	Whole crop silage	Middle of Nov., 2008	May 15-20, 2009	Dough to yellow ripe
B	Tamaizumi	Whole crop silage	Nov. 5, 2008	Jun. 2, 2009	Dough to yellow ripe
C	Nisinokaori	Whole crop silage	Middle of Dec., 2008	May 25, 2009	Dough to yellow ripe
	Norin 61	Whole crop silage	Middle of Dec., 2008	May 25, 2009	Dough to yellow ripe
D	Nishinokaori	Straw silage	Middle of Nov., 2008	Middle to end of Jun., 2009	Matured

Table 2. Fermentation characteristics of whole crop wheat silage and wheat straw silage.

		pH	Dry matter (g kg ⁻¹)	Lactate (g kg ⁻¹ DM)	Acetate (g kg ⁻¹ DM)	L/A ⁷	Butyrate (g kg ⁻¹ DM)	NH ₃ -N (g kg ⁻¹ TN ⁸)	V-score
WCWS ¹ (n=25)	Mean	4.46	363	19.2	10.4	1.43	4.7	71.4	80.6
	Max ³	4.95	434	41.6	16.2	4.02	15.5	117.7	97.7
	Min ⁴	3.81	301	5.3	4.5	0.40	0.0	21.4	44.9
	SD ⁵	0.36	39.0	10.7	3.6	1.05	4.8	2.6	15.7
	CV ⁶ (%)	8.0	10.7	55.7	34.5	74.6	101.8	36.6	19.4
WSS ² (n=10)	Mean	5.46	637	1.9	2.2	1.27	1.0	8.8	95.8
	Max	5.76	732	3.8	5.5	4.92	4.1	26.1	99.7
	Min	4.79	439	0.7	0.1	0.42	0.0	0.0	83.1
	SD	0.35	108	1.1	1.9	1.40	16.3	7.4	6.5
	CV(%)	6.4	16.9	59.9	89.0	110.7	165.1	83.5	6.8

¹ whole crop wheat silage, ² wheat straw silage, ³ maximum, ⁴ minimum, ⁵ standard deviation, ⁶ coefficient of variation, ⁷ molar ratio of lactate and acetate, ⁸ total nitrogen.

to differences of grain maturation and extent of drying up of leaf and stem fractions, while the range of WSS was due to differences of wilting conditions such as ambient temperature and moisture levels of the soil.

The pH of WCWS ranged from 3.8 to 5.0 (average of 4.46). Lactic acid, a main fermented product of ensiling of whole crop wheat, was from 5.3 to 41.6 g kg⁻¹DM. As other acids, the mean of acetic and butyric acids were 10.4 and 4.7 g kg⁻¹DM, respectively. The lower molar ratio of lactate to acetate was also observed in the WCWS that could be implied that hetero-ferment lactic acid bacteria and/or clostridia that ferment lactate to butyrate were relatively active in microflora of WCWS¹⁸. In comparison with WCWS produced in other areas^{6, 8, 19}, they showed lower pH (<4.2) and similar or higher lactate (>2.0 g kg⁻¹DM) than the present study. The fermentation quality in the WCWS considerably varied among the cropping sites and among the bales within the same cropping site as shown by the V-score with ranging from 45 to 98. The significantly positive relationship between DM content and V-score was also observed, showing a single regression equation expressed as $y = 0.2974x - 27.414$ ($r = 0.741$, $P < 0.001$), where y is V-score and x is DM content of WCWS. Since the V-score evaluation can indicate the extent of butyric fermentation and/or protein degradation, that result was consistent with the fact that clostridia are more sensitive to high DM condition than are LAB¹⁸. This implies that the DM content of the original materials should be increased to higher than 360 g kg⁻¹ before ensiling to obtain over 80 of the V-score. The number of epiphyte LAB on the whole crop wheat in some cropping sites was also counted, and the results were less than 10³ colony forming units per g fresh matter of the original material (data not shown), that was lower than the recommended count (about 10⁵ cfu/gFM) for stable fermentation. Addition of LAB inoculants therefore may enhance lactic acid

fermentation in WCWS, in accompany with sufficient soluble carbohydrates.

The compaction density of round-baled WCWS in the present study averaged of 147 kg DM m⁻³ (not shown in tables). This was comparably lower than those of 230 kg DM m⁻³ generally observed with bunker silo, which showed a higher fermentation quality²⁰. Moriya *et al.*²¹ reported lower pH and higher lactate concentration of the silage as it was processed with short-length cutting and high-density baling. The whole crop rice silage was also reported to be considerably influenced for fermentation quality by harvesting and baling treatments^{2, 22}. The whole crop rice and whole crop wheat have higher hollow stem materials. This limit increasing the compaction density, which can induce lower oxygen in the bales and bunker silos and can enhance anaerobic conditions for better growth of lactic acid bacteria.

The WSS had relatively high DM contents, but the contents varied among the bales. That was probably due to differences of the weather condition during the times between grain harvesting and straw baling. The concentrations of lactate, acetate, butyrate, and ammonia in the WSS, however, varied less among bales.

Nutrient composition: The WCWS examined in the present study was characterized of higher NFC and lower NDF constituents as shown by the average of 306 and 546 g kg⁻¹DM, respectively (Table 3). The NFC and NDF contents in the WCWS were also inversely related ($r = -0.683$, $P < 0.001$). This would indicate differences of the grain composition and grain maturation among cropping sites, as shown by the range of NFC composition from 229 to 413 g kg⁻¹DM. Since the three cultivars of winter wheat crop examined in the present study have similar cumulative temperature requirement (875 to 890°C from boot to grain matured stages, as based on local prefectural data), the differences would be

Table 3. Nutrient composition of whole crop wheat silage and wheat straw silage.

		OM	CP	NFC	EE	NDF	ADF	ADL	Hemicellulose	Cellulose
WCWS ¹	Mean (g kg ⁻¹ DM)	953	78	306	24	546	323	42	223	280
	Max ³ (g kg ⁻¹ DM)	967	110	413	49	608	362	54	246	321
	Min ⁴ (g kg ⁻¹ DM)	937	52	229	14	449	276	30	174	240
	SD ⁵	8.9	17.0	39.1	8.7	33.2	19.3	5.6	16.8	16.7
	CV ⁶ (%)	0.9	22.0	12.8	36.1	6.1	6.0	13.3	7.6	5.9
WSS ²	Mean (g kg ⁻¹ DM)	939	19	119	26	775	479	65	296	415
	Max (g kg ⁻¹ DM)	951	21	129	36	816	516	74	314	442
	Min (g kg ⁻¹ DM)	921	17	83	20	749	451	57	282	390
	SD	9.0	1.3	13.6	5.3	18.5	21.6	5.6	10.6	16.3
	CV(%)	1.0	7.0	11.4	20.4	2.4	4.5	8.7	3.6	3.9

¹ whole crop wheat silage, ² wheat straw silage, ³ maximum, ⁴ minimum, ⁵ standard deviation, ⁶ coefficient of variation.

Table 4. *In vitro* rumeninal gas production, DM and OM degradability of whole-crop wheat silage and wheat straw silage.

		IVDMD ⁷	IVOMD ⁸	Gas3h	Gas6h	Gas9h	Gas12h	Gas24h	Gas48h	Gas72h
		(%)	(%)	----- (ml/gDM) -----						
WCWS ¹	Mean	58.2	56.1	24.3	54.9	78.3	96.6	141.9	206.2	229.5
	Max ³	64.8	63.2	34.5	64.0	95.1	115.8	164.2	230.8	253.6
	Min ⁴	48.6	45.1	16.5	46.5	67.5	83.4	120.7	190.2	209.7
	SD ⁵	3.5	3.8	5.5	6.2	7.5	8.8	10.3	10.3	10.2
	CV ⁶ (%)	5.9	6.8	22.5	11.4	9.5	9.1	7.2	5.0	4.4
WSS ²	Mean	33.6	29.3	11.9	22.4	31.0	40.8	63.8	112.1	147.7
	MAX	34.5	30.6	18.3	30.4	39.3	48.1	71.3	123.3	158.2
	MIN	32.9	28.3	7.1	13.6	21.2	32.6	56.5	103.8	141.3
	SD	0.6	0.8	3.8	5.7	6.3	5.3	5.3	7.2	6.5
	CV(%)	1.7	2.6	31.4	25.3	20.3	13.1	8.3	6.4	4.4

¹ whole crop wheat silage, ² wheat straw silage, ³ maximum, ⁴ minimum, ⁵ standard deviation, ⁶ coefficient of variation, ⁷ *in vitro* DM degradability, ⁸ *in vitro* OM degradability.

due to the seeding time, soil condition (moisture, fertilizer) and climate differences after seeding.

The average content of OM, CP and EE in WCWS were 953, 78 and 24 g kg⁻¹DM, respectively. Gross compositions of the WCWS were therefore similar to values of the WCWS reported in Middle East and Europe^{19, 23-25}. The WCWS was observed much preferable to timothy hay, a representative forage of dairy cattle in Japan, which contains CP 70-100, NFC 130-200 and NDF 600-720 g kg⁻¹DM²⁶, and was comparable with whole crop rice silage (WCRS). The WCRS harvested at the dough to yellow ripe stages was reported to have NDF content of 480 to 560, CP content of 60 to 80 and NFC content of 190 to 300 g kg⁻¹DM²⁶.

The WSS had lower contents of CP and NFC and higher contents of cell wall constituents as compared to those of the WCWS. Cellulose fraction (415 g kg⁻¹DM) in the WSS was almost the same to that of the wheat straw previously reported²⁷⁻²⁹, while the NDF content was higher than that of the rice straw (630 g kg⁻¹DM)²⁶.

***In vitro* ruminal degradability and gas production:** The IVDMD and IVOMD of WCWS averaged 58.2% and 56.1%, ranging from 48.6 to 64.8% and from 45.1 to 63.2%, respectively (Table 4). These were lower and more variable than the results of Weinberg *et al.*³⁰, who reported that the IVDMD of WCWS was 67 to 69% at the flowering stage and 65 to 66% at the milk-ripe stage.

Total gas production at *in vitro* rumen incubation of the WCWS was averaged of 141.9, 206.2 and 229.5 ml gDM⁻¹ at 24, 48 and 72 h incubations, respectively. They were comparable or higher than those of timothy hay, which had 121.0, 167.5 and 183.0 ml gDM⁻¹ at 24, 48 and 72 h, respectively. The rate of gas production of WCWS was higher at the first 24 h incubation, as shown by averages of 8.1 ml h⁻¹ at the first 24 h, 2.7 ml h⁻¹ at 24 to 48h, and 1.0 ml h⁻¹ at 48 to 72 h incubation. This was consistent with the general observation that starch and soluble sugars in feeds, which are more degradable components, have higher rate of rumen fermentation^{31,32}. The rumen fermentation is therefore related with grain composition and maturation

of the wheat crop as these relates to the contents of these soluble carbohydrates. The higher coefficient of variation for gas production at the initial stages of *in vitro* rumen incubation was also probably associated with the observation of higher coefficient of variation for NFC content as compared to those of fiber fractions such as NDF and ADF.

The WSS, only containing straw fractions, was certainly less degraded showing averaged values of 33.6% and 29.6% for the IVDMD and IVOMD, respectively, which were almost a half of the WCWS. The IVOMD of WSS in the present study was comparative to the value of 24% reported by Tang *et al.*²⁷.

Correlation of silage fermentation characteristics, nutrient composition and ruminal degradability of WCWS: Parameters of fermentation characteristics of silage (pH, lactate and acetate) were not significantly correlated to *in vitro* ruminal degradation (Table 5). The V-score showed significant positive relationship with the IVDMD and IVOMD (P<0.05). The concentrations of butyrate and ammonia in the WCWS were also inversely correlated with the IVDMD and IVOMD (P<0.05). Since significantly positive correlations between NFC content in the WCWS and the *in vitro* rumen degradability (P<0.0) was observed, it was certain that soluble carbohydrates and starch in the WCWS contributed to the better conditions both for ensiling process and for microbial digestion in the rumen.

The IVOMD and IVDMD were inversely correlated with contents of NDF (P<0.001), ADF (P<0.001), ADL (P<0.05), hemicellulose (P<0.01) and cellulose (P<0.01, Table 6). This was consistent with the general observation for the degradability of feed crops including maize, sorghum, and small grain crops^{18,27}. McGeough *et al.*²⁷ reported that whole crop wheat had a greater degradability with advancing from the growing stage to the grain-matured stage, due to higher contents of starch and soluble carbohydrates and lower contents of cell wall constituents. There were thus no relationships of contents of OM, CP, and EE with the *in vitro* rumen degradability.

Table 5. Correlation coefficient (*r*) of fermentation characteristics and *in vitro* ruminal gas production, *in vitro* dry matter and organic matter degradability of whole crop wheat silage.

	pH	Dry matter	Lactate	Acetate	L/A ³	Butyrate	NH ₃ -N/TN ⁴	V-score
IVDMD ¹	-0.139	0.302	0.127	-0.198	0.175	-0.445*	-0.352	0.481*
IVOMD ²	-0.206	0.369	0.172	-0.232	0.223	-0.510**	-0.397*	0.543**
Gas3h	0.122	0.284	-0.213	-0.487*	0.245	-0.264	0.003	0.239
Gas6h	0.146	0.471*	-0.181	-0.595**	0.223	-0.331	-0.311	0.387
Gas9h	0.006	0.338	0.060	-0.312	0.168	-0.271	-0.446*	0.367
Gas12h	-0.058	0.281	0.122	-0.173	0.105	-0.258	-0.429*	0.341
Gas24h	0.163	-0.039	0.022	-0.069	0.090	-0.002	-0.110	0.062
Gas48h	0.109	0.229	-0.100	-0.232	0.024	-0.246	-0.211	0.282
Gas72h	0.058	0.412*	-0.122	-0.380	0.075	-0.419*	-0.284	0.438*

¹ *in vitro* DM degradability, ² *in vitro* OM degradability, ³ molar ratio of lactate and acetate, ⁴ Total nitrogen. * P<0.05, ** P<0.01.

Table 6. Correlation coefficient (*r*) of nutritional composition and *in vitro* ruminal gas production, *in vitro* dry matter and organic matter degradability of whole crop wheat silage.

	OM	CP	NFC	EE	NDF	ADF	ADL	Hemicellulose	Cellulose
IVDMD ¹	0.366	-0.078	0.723***	-0.070	-0.692***	-0.681***	-0.508**	-0.588**	-0.617***
IVOMD ²	0.455*	-0.149	0.754***	-0.117	-0.655***	-0.647***	-0.454*	-0.554**	-0.596**
Gas3h	0.243	-0.192	0.191	-0.319	0.026	0.042	-0.061	-0.019	0.088
Gas6h	0.228	-0.115	0.443*	-0.196	-0.354	-0.347	-0.230	-0.289	-0.323
Gas9h	0.203	0.101	0.543**	-0.010	-0.632***	-0.657***	-0.264	-0.496*	-0.673***
Gas12h	0.202	0.114	0.584**	-0.042	-0.679***	-0.729***	-0.291	-0.504*	-0.747***
Gas24h	-0.041	0.465*	0.424*	0.106	-0.775***	-0.780***	-0.363	-0.636**	-0.781***
Gas48h	0.189	0.101	0.692***	-0.117	-0.782***	-0.787***	-0.410*	-0.641***	-0.775***
Gas72h	0.341	-0.124	0.764***	-0.194	-0.691***	-0.667***	-0.371	-0.600**	-0.648***

¹ *in vitro* DM degradability ² *in vitro* OM degradability. *P<0.05; **P<0.01, ***P<0.001.

Conclusions
The winter cropping of wheat plant as whole crop silage at the post-rice cropping can supply fermentable energy in rumen. The wheat straw also can be useful as fibrous material by ensiling. However, the fermentation quality and nutritional values of the silage considerably varied, indicating

associative effects of agronomic residue, soil condition and harvesting time. Further research is required to elucidate the effect of seeding and harvesting time (maturing stage) on fermentation characteristics and nutritive value of WCWS in a double cropping system with rice for food and forage.

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