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The determination of in vitro gas production and metabolizable energy value of rice straw treated with exogenous fibrolytic enzymes

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Abstract: This study was conducted to determine the effects of cellulase and xylanase treatments on in vitro 24-h gas production (GP), organic matter digestibility (OMD), and metabolizable energy (ME) values of rice straw (RS). Rice straws were treated with cellulase and xylanase at the levels of 0% (C1), 0.5% (RS+CEL0.5), 1% (RS+CEL1), 1.5% (RS+CEL1.5), and 2% (RS+CEL2) and 0% (C2), 0.5% (RS+XYL0.5), 1% (RS+XYL1), 1.5% (RS+XYL1.5), and 2% (RS+XYL2), respectively. Rice straws treated with each level of cellulase and xylanase were ensiled in 6 glass jars. For each level of cellulase and xylanase treatment, 3 glass jars were incubated at room temperature, while 3 glass jars were incubated at 40 ± 0.2 °C in an incubator for 30 days. In vitro GP for 24 h, OMD, and ME values in RS+CEL2 were higher ($P < 0.05$) than those of C1 and the other groups treated with cellulase, while significant increases ($P < 0.05$) for the same parameters were obtained from RS+XYL1 incubated at 40 ± 0.2 °C, RS+XYL1.5, and RS+XYL2 compared to C2, RS+XYL0.5, and RS+XYL1 incubated at room temperature. In conclusion, in vitro 24-h GP, OMD, and ME values were the highest ($P < 0.05$) in the groups treated with the highest level of cellulase and xylanase incubated at 40 ± 0.2 °C.

Key words: Fibrolytic enzyme, in vitro gas production, metabolizable energy, rice straw

1. Introduction

Rice straw (RS) is a crop residue of rice production and can be used as a feed for ruminants because it is easily and cheaply available in many Asian countries. Kadam et al. (1) stated that about 1.35 metric tons of RS are left for each metric ton of harvested rice. Rice straw, such as other cereal straws, does not provide enough nutrients to meet the requirement of ruminants due to its high lignocellulosic structure, relatively low digestibility, and poor palatability (2). Average dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin contents of different varieties of RS range from 92.21% to 93.05%, 81.21% to 86.24%, 3.49% to 5.10%, 72.16% to 77.57%, 41.38% to 46.32%, and 4.3% to 6.97%, respectively (3).

Low quality forages (straws) can be treated with different physical methods (grinding, pelleting/chopping for reducing particle size, soaking, steam treatments, cooking under pressure, etc.) to improve their feeding value. Likewise, chemical treatment of low-quality forages improves their degradability (4); however, the chemicals may result in environmental pollution. Thus, the interest in treatment of straws is focused on biological methods owing to the ongoing concerns about pollution and food safety. Exogenous enzymes obtained from fungi and bacteria can be used in biological treatment to improve the digestibility of low-quality forages due to being able to break down lignocellulosic complexes (5). Improving the availability of the nutrients in the cell wall structure of fibrous feeds can be possible by using exogenous fibrolytic enzymes. These enzymes can be directly added to feeds before feeding or during ensiling (6). It was reported that fibrolytic enzymes have effects on the degradation of cereal straw cell wall and improvement of the nutritive value of cereal straws (7,8).

The aim of the present study was to determine the effects of different levels of cellulase and xylanase treatments of RS on in vitro 24-h gas production (GP), organic matter digestibility (OMD), and metabolizable energy (ME) values.

2. Materials and methods

Chemical analyses and in vitro gas production techniques were conducted in the Ruminant Feed Evaluation

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2.1. Enzyme treatments

Rice straw was chopped to 5 cm in length and treated with the cellulase (from *Trichoderma reesei* with activity ≥5000 U/kg; 1 unit of cellulase activity is described as the enzyme required to release 1 μg of reducing sugar from 4 mg/mL sodium cellulose glycolate in 1 min at 37 °C and pH 5.5) and xylanase (from *Bacillus subtilis* with activity ≥100,000 U/kg; 1 unit of xylanase activity is described as the enzyme required to release 1 μmol xylan from xylan solution in 1 min at 37 °C and pH 5.5) at the levels of 0% (C1), 0.5% (RS+CEL0.5), 1% (RS+CEL1), 1.5% (RS+CEL1.5), and 2.0% (RS+CEL2) and 0% (C2), 0.5% (RS+XYL0.5), 1% (RS+XYL1), 1.5% (RS+XYL1.5), and 2.0% (RS+XYL2) of DM basis of RS according to the method reported by Nakashima et al. (9), respectively. The amount of water added to RS for cellulase and xylanase treatments was calculated to provide approximately 40% DM of RS. For ensuring homogeneous distribution of cellulase and xylanase enzymes in RS, the enzymes were dissolved in water and then were immediately sprayed on RS and mixed for absorption by the RS. After mixing, 6 glass jars with a volume of 1 L were filled completely with RS treated with cellulase and xylanase, for each level of cellulase and xylanase treatment. The perimeters of the jar lids were coated with silicone to prevent air intake. For each level of cellulase and xylanase treatments, 3 glass jars were incubated at room temperature (22 °C) in the dark for 30 days and 3 glass jars were incubated at 40 ± 0.2 °C in an incubator for 30 days. After incubation of the jars containing RS treated with cellulase and xylanase, all jars were opened and dried at 100 ± 0.2 °C in an incubator to stop the enzyme activities. Dried RS samples were ground for the in vitro gas production technique and chemical analysis.

2.2. Ruminal fluid collection

Ruminal fluid was obtained from different sites within the rumen of three ruminally cannulated Karayaka rams (average weight 50 ± 5 kg; 2 years old) fed twice daily at 0800 and 1700 hours with a diet containing 650 g of alfalfa hay and 350 g of concentrate estimated to the level of 1.25 × maintenance requirements according to the NRC (10). Fresh drinking water was freely available. Ruminal fluid was collected at 3 h after the morning feeding in thermos flasks and transported directly to the laboratory. Ruminal fluid was strained through four layers of cheesecloth and held at 39 °C under CO_2 atmosphere.

2.3. Chemical analysis

Rice straw samples were milled for passing through a 1 mm sieve and then DM, ash, ether extract (EE), and CP of RS samples were analyzed according to AOAC methods (11). ADF, NDF (with sodium sulfite), and ADL contents of RS samples were determined by the method of Van Soest et al. (12).

2.4. In vitro gas production

In vitro gas production was measured using the ANKOM^{RF} gas production system (13). Each unit of the system consisted of a 250 mL glass bottle equipped with an attached module. There was wireless communication between the attached module and computer. When the pressure inside of each unit reached 1.5 kPa above ambient pressure, a valve of the attached module automatically released the gas accumulated in the headspace of the glass bottles. Pressure inside of the glass bottles was measured every 10 min. The buffer solution was prepared according to Menke and Steingass (13), preheated, and mixed with rumen fluid at 4:1, and then 100 mL of buffered rumen fluid was added to each preheated glass bottle containing 1 g of the milled feed samples. The incubation media preparation was carried out under anaerobic conditions purged with CO_2, at approximately 39 °C and pH of about 6.5–6.8. After filling, all glass bottles were closed and placed into an incubator at 39.0 ± 0.5 °C. The incubation procedure was conducted in triplicate for each treatment group. All glass bottles containing incubation medium and feed samples were incubated for 0, 3, 6, 12, 24, 48, 72, and 96 h. The average cumulative pressure measured for each incubation time of the feed samples and ME (MJ/kg DM) and OMD (%) values of RS samples were estimated from the measured pressure by in vitro gas production method at 24 h by using the following equations (14).

$$ME\ (MJ/kg\ DM) = 2.20 + 0.1136GP + 0.0057CP + 0.00029EE^2$$

$$OMD\ (\%) = 57.2 + 0.365GP + 0.304CP - 1.98ADL$$

2.5. Statistical analysis

Data were designed for appropriate one-way classification with a 2 × 5 factorial experimental design (full factorial design model) and analyzed with the GLM procedure. Data were fitted to the statistical model with a full factorial design including main effects and all possible interaction effects of the factor combinations (enzyme treatments), temperature, and levels of the traits due to selecting for the reducing model. Therefore, a mathematical model including three main effects and only traits × levels interaction effects was fitted to data due to the fact that the other interaction effects were insignificant. Main effects were compared with Duncan's multiple range test. Mean differences of interaction effects were compared by Tukey test. All analyses and calculations were performed with SAS (15).

3. Results

Dry matter, ash, CP, ADF, NDF, and ADL contents of rice straw used in the study were 93.53%, 16.68%, 4.15%,

41.15%, 65.72%, and 7.85%, respectively. Cumulative GP for 24 h, OMD%, and ME (MJ/kg DM) values of RS untreated and treated with different levels of cellulase and xylanase enzymes and incubated at room temperature (22 °C) and 40 ± 0.2 °C for 30 days are given in Tables 1 and 2, respectively. Least square of means and standard errors of means for interaction effects on the traits × levels are presented in Table 3. There were differences ($P < 0.05$) in cumulative GP for 24 h, OMD%, and ME (MJ/kg DM) values of groups treated with the increasing level of cellulase enzyme and incubated at 40 ± 0.2 °C for 30 days (Table 1). Cumulative GP for 24 h, OMD%, and ME (MJ/kg DM) values of RS+XYL1, RS+XYL1.5, and RS+XYL2 incubated at 40 ± 0.2 °C were higher ($P < 0.05$) than those of C2 and RS+XYL0.5 incubated at the same incubation temperature (Tables 2 and 3).

Cumulative gas production values for 3, 6, 12, 24, 48, 72, and 96 h of RS untreated and treated with different levels of cellulase and xylanase enzymes and incubated at room temperature (22 °C) and 40 ± 0.2 °C for 30 days are given in Table 4.

4. Discussion

Plant cell wall components are broken down with fibrolytic enzymes such as cellulase and xylanase produced by microorganisms in the reticulorumen of ruminants. However, many forages like cereal straws are of low quality due to poor digestibility and limited energy value for ruminants (16) because of insufficient degradation of the cell wall in the rumen. The use of exogenous enzymes may be an option to increase the degradability of cell wall fraction (17). Fibrolytic enzymes as feed additives, added in liquid or granular form to diets, hay, silages, etc. to increase the availability of nutrients in the plant cell wall may be obtained from microbial fermentation of bacteria or fungi, and they show specific activity. Exogenous fibrolytic enzymes applied both during feed ensiling and directly during feeding increased in vitro GP and improved the availability of nutrients of fibrous feeds. These supplementations may allow the attachment of microorganisms to feeds (18) and/or the formation of enzyme–feed complexes (19). Beauchemin et al. (16) stated that exogenous enzyme treatments in a liquid form of dried feeds were important for adsorption of enzymes to provide suitable attachment to feeds before consumption. In the present study, 24-h GP, OMD%, and ME values of the RS+CEL1, RS+CEL1.5, RS+CEL2, RS+XYL1.5, and RS+XYL2 groups treated with cellulase and xylanase dissolved in water and sprayed on rice straw and incubated at room temperature (22 °C) for 30 days improved compared to those of controls and other treatment groups, while the same parameters increased in RS+CEL0.5, RS+CEL1, RS+CEL1.5, RS+CEL2, RS+XYL1, RS+XYL1.5, and RS+XYL2 incubated at 40 ± 0.2 °C for 30 days compared to those of controls.

Kumar et al. (20) stated that some commercial fibrolytic enzymes had effects on increasing of total GP of feeds. Akinfemi and Ogunwole (21) reported that 24-h GP, ME, and OMD values increased in RS treated with different mushroom species. In the present study, treatments of RS

Table 1. Cumulative gas production for 24 h, the percentage of OMD, and ME values of rice straw untreated and treated with cellulase and incubated at room temperature and 40 °C for 30 days.

Treatments	Incubations	GP, mL Mean ± SE	OMD% Mean ± SE	ME, MJ/kg DM Mean ± SE
C1	22 °C	28.32 ± 0.48^{f}	53.25 ± 0.17^{f}	6.29 ± 0.06^{f}
	40 °C	28.60 ± 0.31^{f}	53.36 ± 0.31^{f}	6.33 ± 0.04^{f}
RS+CEL0.5	22 °C	29.32 ± 0.28^{f}	53.62 ± 0.10^{f}	6.42 ± 0.03^{f}
	40 °C	30.63 ± 0.43^{e}	54.09 ± 0.15^{e}	6.60 ± 0.05^{e}
RS+CEL1	22 °C	31.53 ± 0.48^{de}	54.42 ± 0.17^{de}	6.72 ± 0.06^{de}
	40 °C	32.11 ± 0.08^{cd}	54.63 ± 0.03^{cd}	6.80 ± 0.01^{cd}
RS+CEL1.5	22 °C	31.73 ± 0.21^{cd}	54.50 ± 0.07^{cd}	6.75 ± 0.02^{cd}
	40 °C	32.68 ± 0.29^{bc}	54.84 ± 0.10^{bc}	6.88 ± 0.03^{bc}
RS+CEL2	22 °C	33.20 ± 0.20^{b}	55.03 ± 0.07^{b}	6.95 ± 0.02^{b}
	40 °C	34.56 ± 0.29^{a}	55.53 ± 0.10^{a}	7.14 ± 0.04^{ba}

Different letters within the same column indicate differences among groups ($P < 0.05$).
GP: Gas production; OMD: organic matter digestibility; ME: metabolizable energy; DM: dry matter; C1: control; RS+CEL0.5: rice straw+cellulase 0.5%; RS+CEL1: rice straw+cellulase 1%; RS+CEL1.5: rice straw+cellulase 1.5%; RS+CEL2: rice straw+cellulase 2%.

Table 2. Cumulative gas production for 24 h, the percentage of OMD, and ME values of rice straw untreated and treated with xylanase and incubated at room temperature and 40 °C for 30 days.

Treatments	Incubations	GP, mL Mean ± SE	OMD% Mean ± SE	ME, MJ/kg DM Mean ± SE
C2	22 °C	28.32 ± 0.48^{d}	53.25 ± 0.17^{d}	6.29 ± 0.06^{d}
	40 °C	28.60 ± 0.31^{d}	53.36 ± 0.31^{d}	6.33 ± 0.04^{d}
RS+XYL0.5	22 °C	28.85 ± 0.31^{d}	53.44 ± 0.11^{d}	6.36 ± 0.04^{d}
	40 °C	29.00 ± 0.20^{d}	53.50 ± 0.07^{d}	6.38 ± 0.02^{d}
RS+XYL1	22 °C	29.29 ± 0.45^{d}	53.60 ± 0.16^{d}	6.42 ± 0.06^{d}
	40 °C	30.69 ± 0.35^{c}	54.12 ± 0.13^{c}	6.61 ± 0.04^{c}
RS+XYL1.5	22 °C	31.39 ± 0.12^{c}	54.37 ± 0.04^{c}	6.71 ± 0.01^{c}
	40 °C	33.04 ± 0.26^{b}	54.97 ± 0.09^{b}	6.93 ± 0.03^{b}
RS+XYL2	22 °C	33.65 ± 0.25^{ab}	55.20 ± 0.09^{ab}	7.01 ± 0.03^{ab}
	40 °C	34.18 ± 0.16^{a}	55.39 ± 0.06^{a}	7.08 ± 0.02^{a}

Different letters within the same column indicate differences among groups ($P < 0.05$).
GP: Gas production; OMD: organic matter digestibility; ME: metabolizable energy; DM: dry matter; C2: control, RS+XYL0.5: rice straw+xylanase 0.5%; RS+XYL1: rice straw+xylanase 1%; RS+XYL1.5: rice straw+xylanase 1.5%; RS+XYL2: rice straw+xylanase 2%.

Table 3. Least square of means and standard error of means for interaction effects on traits × levels.

		Traits		
Factors	Levels	GP 24-h, mL	OMD, %	ME, MJ/kg DM
C	0	28.46 ± 0.23a	53.30 ± 0.07a	6.31 ± 0.031a
RS+CEL	0.5	29.97 ± 0.23b	53.86 ± 0.10a	6.51 ± 0.031b
	1	31.82 ± 0.23c	54.53 ± 0.10b	6.76 ± 0.031b
	1.5	32.21 ± 0.23c	54.67 ± 0.10b	6.82 ± 0.031b
	2.0	33.88 ± 0.23d	55.28 ± 0.10c	7.04 ± 0.031d
RS+XYL	0.5	28.92 ± 0.23a	53.47 ± 0.10a	6.37 ± 0.031a
	1	29.99 ± 0.23b	53.87 ± 0.10a	6.51 ± 0.031b
	1.5	32.21 ± 0.23c	54.67 ± 0.10b	6.82 ± 0.031c
	2.0	33.91 ± 0.23d	55.29 ± 0.10bc	7.05 ± 0.031d
P-value		0.0003	0.006	0.0003

GP 24-h: Gas production for 24 h; OMD: organic matter digestibility; ME: metabolizable energy; DM: dry matter; C: control, RS+CEL: rice straw+cellulase; RS+XYL: rice straw+xylanase.

with increasing levels of cellulase significantly increased 24-h GP, ME, and OMD of the RS+CEL1, RS+CEL1.5 and RS+CEL2 groups incubated at two different incubation temperatures for 30 days compared to those of control and RS+CEL0.5 groups incubated at room temperature. Similar increases in the same parameters were obtained from RS+XYL1 incubated at 40 ± 0.2 °C and RS+XYL1.5 and RS+XYL2 groups incubated at two different temperatures for 30 days.

Tang et al. (22) reported that fibrolytic enzyme supplementations improved the in vitro OMD of RS. OMDs of treated rice straws varied from 53.62 ± 0.10% to 55.53 ± 0.10% for cellulase treatments and from 53.44 ± 0.11% to 55.39 ± 0.06% for xylanase treatments in the present study.

Although gas production, basically the result of rumen fermentation, seems like a loss of energy in terms of nutrition, it has been used to predict OMD and ME

Table 4. Cumulative gas production values (mean ± SE) of rice straw untreated and treated with cellulase or xylanase.

Cumulative gas production, mL									
Treatments		Levels, %	3 h	6 h	12 h	24 h	48 h	72 h	96 h
C	22 °C	0	3.42 ± 0.14	7.76 ± 0.22	11.39 ± 0.14	28.32 ± 0.48	31.99 ± 0.35	39.37 ± 0.2	41.64 ± 0.10
	40 °C	0	3.38 ± 0.14	7.84 ± 0.10	11.68 ± 0.21	28.60 ± 0.31	32.11 ± 0.43	39.54 ± 0.21	42.16 ± 0.10
RS+CEL	22 °C	0.5	3.21 ± 0.07	7.92 ± 0.07	11.51 ± 0.14	29.32 ± 0.28	33.35 ± 0.10	39.62 ± 0.21	41.51 ± 0.25
		1.0	3.59 ± 0.07	11.59 ± 0.27	25.38 ± 0.53	31.53 ± 0.48	35.70 ± 0.25	43.09 ± 0.18	44.87 ± 0.17
		1.5	4.76 ± 0.09	13.99 ± 0.14	28.81 ± 0.17	31.73 ± 0.21	37.23 ± 0.14	43.09 ± 0.07	45.52 ± 0.22
		2.0	5.65 ± 0.10	14.86 ± 0.28	27.24 ± 0.58	33.20 ± 0.20	43.09 ± 0.18	50.60 ± 0.50	53.04 ± 0.21
	40 °C	0.5	3.46 ± 0.14	8.50 ± 0.17	11.88 ± 0.14	30.63 ± 0.43	33.18 ± 0.37	39.70 ± 0.17	42.39 ± 0.10
		1.0	4.25 ± 0.10	13.08 ± 0.25	27.73 ± 0.35	32.11 ± 0.08	36.86 ± 0.14	43.83 ± 0.14	45.68 ± 0.25
		1.5	5.40 ± 0.10	14.98 ± 0.21	30.15 ± 0.10	32.68 ± 0.29	39.03 ± 0.14	44.95 ± 0.32	47.01 ± 0.10
		2.0	6.48 ± 0.21	16.88 ± 0.10	28.77 ± 0.32	34.56 ± 0.29	44.16 ± 0.14	52.42 ± 0.17	54.85 ± 0.21
RS+XYL	22 °C	0.5	3.62 ± 0.21	9.12 ± 0.10	14.73 ± 0.14	28.85 ± 0.31	32.27 ± 0.32	39.04 ± 0.35	41.25 ± 0.18
		1.0	3.54 ± 0.17	11.66 ± 0.18	23.52 ± 0.28	29.29 ± 0.45	32.77 ± 0.21	40.03 ± 0.17	41.89 ± 0.28
		1.5	4.62 ± 0.14	13.12 ± 0.28	28.85 ± 0.25	31.39 ± 0.12	35.12 ± 0.17	41.15 ± 0.10	43.09 ± 0.14
		2.0	5.64 ± 0.17	13.47 ± 0.16	25.26 ± 0.32	33.65 ± 0.25	42.47 ± 0.28	51.39 ± 0.32	53.24 ± 0.14
	40 °C	0.5	4.08 ± 0.13	9.32 ± 0.10	15.14 ± 0.14	29.00 ± 0.20	33.22 ± 0.14	40.08 ± 0.10	42.23 ± 0.14
		1.0	4.21 ± 0.14	12.49 ± 0.20	24.51 ± 0.21	30.69 ± 0.35	33.53 ± 0.07	40.47 ± 0.16	42.59 ± 0.07
		1.5	5.77 ± 0.17	14.69 ± 0.17	29.88 ± 0.21	33.04 ± 0.27	35.87 ± 0.10	41.89 ± 0.21	43.87 ± 0.14
		2.0	6.76 ± 0.17	14.90 ± 0.28	27.03 ± 0.14	34.18 ± 0.16	44.45 ± 0.25	53.08 ± 0.21	55.14 ± 0.14

C: Control; RS+CEL; rice straw+cellulase; RS+XYL: rice straw+xylanase.

values of feeds. Therefore, the increase in the amount of gas production due to the increase in the activity of rumen microorganisms causes an increase in both OMD and ME values. In this study, digestible organic matter and ME values of RS+CEL2 and RS+XYL2 were higher than those of control. Sommart et al. (23) and Nitipot and Sommart (24) stated that there was a positive correlation between in vitro OMD and the volume of gas released during fermentation.

Jalc (5) reported that usage of exogenous enzymes obtained from fungi, white rot fungi, and mushroom has a potential effect on improving the digestibility of low-quality forages due to being able to break down lignocellulosic complex. Some toxic metabolites may be produced by the fungi during the treatment for increasing feeding value of rice straw. However, treatment of the straws with fungi is difficult in terms of ensuring optimal conditions (pH, temperature, CO_2 concentration, etc.) for fungal growth. Hence, commercial products will be important in the future for ruminant nutrition (16). Fazaeli et al. (25) and Rodrigues et al. (26) reported that enzyme treatment or its combination with other treatments has effects on increasing the digestibility of cereal straws for ruminants.

It was stated that treatment with cellulase (27) and cellulase and xylanase (28) improved the digestibility of RS. In the present study, treatments of rice straw with increasing levels of cellulase and xylanase significantly improved OMD compared to untreated rice straw. This result was consistent with the statements of some other researchers (25,26,28). OMD was the highest in RS+CEL2 and RS+XYL incubated at 40 ± 0.2 °C for 30 days. This result can be attributed to the cellulase and xylanase levels and their activity resulting in changes in cell wall structure in incubation.

In conclusion, cellulase and xylanase treatments of rice straw had higher 24-h GP, OMD, and ME values compared to untreated rice straw. The best result was obtained with the highest level of cellulase (RS+CEL2) and xylanase (RS+XYL2) treatments at 40 ± 0.2 °C when in vitro 24-h GP, OMD, and ME values were considered.

Although fibrolytic enzyme treatments improved the 24-h GP, OMD, and ME values, further low-quality forage treatment experiments with exogenous enzymes alone and in combinations are required to improve digestibility. The findings of in vitro studies should also be supported by the results of in vivo studies.

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