Research Article

Effects of Supplementation of Exogenous Fibrolytic Enzyme Enhances on Gas Production, Digestibility and Rumen Fermentation of Rice Straw by Using *In vitro* Gas Production Technique

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Abstract

This study was conducted by using an *in vitro* gas production technique to study the level of enzyme supplements. Dietary treatments were total mixed ration without (control) and with exogenous fibrolytic enzyme (EFE) supplementation at 250, 500 and 750 mg/kg DM, respectively. The *in vitro* dry matter digestibility (IVDMD), *In vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), ammonia nitrogen (NH_3-N), total digestible nutrient (TDN), the partitioning factor of incubation (PF) and microbial crude protein (MCP) at 24 h. after incubation of EFE supplementation at 500mg/kg DM diets was higher than control group and EFE supplementation at 250 and 750mg/kg. This result confirmed that EFE supplementation at 500 mg/kg DM was the suitable level to enhance fiber degradation and improve energy density and end product of fermentation of TMR with 50% rice straw and 50% concentrate.

Keywords: Exogenous fibrolytic enzyme, Rice straw and *In vitro* gas production technique

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ผลของการเสริมเอนไซม์ย่อยเยื่อใยจากภายนอกเพื่อเพิ่มปริมาณการผลิตแก๊ส ประสิทธิภาพการย่อยได้และกระบวนการหมักในกระเพาะรูเมนของฟางข้าว โดยเทคนิค *in vitro* gas production technique

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บทคัดย่อ

การศึกษาครั้งนี้ได้ดำเนินการโดยใช้เทคนิค *in vitro* gas production technique เพื่อศึกษาระดับเสริมเอนไซม์ย่อยเยื่อใยจากภายนอกโดยอาหารทดลองเป็นอาหารผสมสำเร็จที่ไม่มีการเสริม (ควบคุม) และเสริมเอนไซม์ย่อยเยื่อใยจากภายนอก (EFE) ที่ระดับ 250, 500 และ 750 มิลลิกรัม/กิโลกรัมอาหาร ตามลำดับ สัมประสิทธิ์การย่อยได้ของวัตถุแห้ง สัมประสิทธิ์การย่อยได้ของอินทรียวัตถุ พลังงานที่ใช้ประโยชน์ได้ (ME) แอมโมเนียไนโตรเจน (NH_3-N) โภชนะที่ย่อยได้รวม (TDN) การใช้ประโยชน์อาหารจากการบ่ม (PF) และจุลินทรีย์โปรตีน (MCP) ณ เวลา 24 ชั่วโมง หลังการบ่มของการเสริมเอนไซม์ย่อยเยื่อใยจากภายนอกที่ระดับ 500 มิลลิกรัม/กิโลกรัมอาหาร สูงกว่ากลุ่มควบคุมและอาหารเสริมเอนไซม์ย่อยเยื่อใยจากภายนอกที่ระดับ 250 และ 750 มิลลิกรัม/กิโลกรัมอาหาร ซึ่งยืนยันว่าการเสริมเอนไซม์ย่อยเยื่อใยจากภายนอกที่ระดับ 500 มิลลิกรัม/กิโลกรัมอาหาร เป็นระดับที่เหมาะสมในการเพิ่มความสามารถในการย่อยสลายเยื่อใยและเพิ่มความหนาแน่นของพลังงานและผลผลิตจากระบวนการหมักของอาหารผสมสำเร็จที่ประกอบด้วยฟางข้าวและอาหารข้นในสัดส่วน 50:50

คำสำคัญ: เอนไซม์ย่อยเยื่อใยจากภายนอก ฟางข้าว และ *in vitro* gas production technique

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Introduction

Tropical feed resources, predominantly low-quality roughages and agricultural crop-residues are of major importance for ruminants. These feedstuffs show significant relationships with rumen microbial ecology and rumen fermentation arrangements (Wora-anu *et al.*, 2007). Animal feed ingredients could deep impact on ruminal fermentation, specifically the roughage sources, their physical form and relevance of microbial end-product (Wanapat *et al.*, 2000). Rice straw is main roughage source for ruminants in Thailand. Generally, rice straw is low in protein, energy and low digestibility. Structural carbohydrates are those that are resistant to the ruminant's digestive enzymes. Neutral detergent fiber (NDF) is fewer digestible than non-fiber carbohydrates (Safari *et al*., 2011); therefore, NDF content in animal feeds or diets is strongly negatively correlated on the release rate of nutrients. Due to low digestibility of NDF by ruminants, amount of forage intake have been limited by NDF content in roughage. Concentrate were used to full fill the energy requirement in ruminants. However, presently, energy sources (cassava chip and corn) were used by another industry, ethanol, together and costs of energy sources were higher. Increasing the proficiency with which the ruminal micro biota degrades roughage significance to increase energy available and decrease feed cost.

Several previous studies showed that exogenous fibrolytic enzymes have been used to improve the nutritive value of fiber-rich diets and the performance of cattle (Kung *et al*., 2000; Elwakeel *et al*., 2007), sheep (Cruywagen and van Zyl, 2008), and goats (Titi, 2003). Moreover, supplementation of exogenous fibrolytic enzymes (cellulase and xylanase) resulting to increase voluntary feed intake, digestibility of nutrient content, ruminal fermentation and animal production performance in ruminants. Therefore, the objectives of this trial will be investigated the use of exogenous fibrolytic enzyme (EFE) to improve feed efficiency of rice straw in ruminants.

Methodology

This experiment was operated by using an *in vitro* gas technique at several incubation times. The experimental design was conducted by using Completely Randomized Design (CRD). Basal diet was 50:50 rice straw and concentrate for total mixed ration (TMR) fed. Four dietary treatments were diets without exogenous fibrolytic enzyme supplementation (control) and with exogenous fibrolytic enzyme supplementation at 250, 500 and 750 g/1,000 kg DM, respectively.

The feed samples (200 mg) were incubated in 40 ml serum bottles essentially by the procedure of Makkar *et al.* (1995). The feedstuffs with and without exogenous fibrolytic enzymes agents were incubated in triplicate. The rumen fluid and particulate matter were collected before the morning feed from two dairy cattle fed on a roughage diet, homogenized, strained and filtered through four layers of cheese cloth. The glassware used

was kept at approximately 39°C and flushed with CO_2 before use. The rumen fluid (660 ml) was added to warm (about 39°C) and reduced medium consisting of 1095 ml distilled water, 730 ml rumen buffer solution (35.0 g $NaHCO_3$ and 4 g NH_4HCO_3 made up to 1 litre with distilled water), 365 ml macro-mineral solution (6.2 g KH_2PO_4, 5.7 g Na_2HPO_4, 2.22 g NaCl and 0.6 g $MgSO_4 \cdot 7H_2O$ made up to 1 litre with distilled water), 0.23 ml micro-mineral solution (10.0 g $MnCl_2 \cdot 4H_2O$, 13.2 g $CaC1_2 \cdot 2H_2O$, 1 g $CoCl_2 \cdot 6H_2O$, 8.0 g $FeC1_2 \cdot 6H_2O$ and made up to 100 ml with distilled water), 1 ml resazurine (01 g made up to 100 ml with distilled water) and 60 ml freshly prepared reduction solution containing 580 mg $Na_2S \cdot 9H_2O$ and 3.7 ml 1 M-NaOH. The mixture was kept stirred under CO_2 at 39°C using a magnetic stirrer fitted with a hot plate. A portion (40 ml) of the rumen-fluid medium was transferred into each syringe and incubated in incubator at 39°C.

Dietary treatments were analyzed for DM and Ash using the procedure of AOAC (1997), NDF and ADF according to Van Soest *et al.* (1991).

The gas production was recorded at 0, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h of incubation. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follow

$$Y = a + b (1 - e^{ct})$$

where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production, y = gas production at time 't'. At 24 and 48 h post inoculation a set of sample was determined *in vitro* true digestibility according to Van Soest and Robertson (1985). The true digestibility was used to calculate microbial mass according to the method of Blummel *et al.* (1997)

Metabolizable energy (ME, MCal/kg DM), the partitioning factor of incubation (PF), gas yield (GY24), short chain fatty acid concentrations (SCFA) and microbial crude protein (MCP) biomass production were mentioned in Salem *et al.* (2015). Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (IVOMD, g/kg OM) were estimated according to Menke *et al.* (1979) as: ME=2.20+0.136GP (mL/0.5 g DM) + 0.057CP (g/kg DM), where, GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

The partitioning factor of incubation (PF; a measure of fermentation efficiency) was calculated as the ratio of DM degradability *in vitro* (DMD, mg) to the volume (mL) of GP at 72 h according to Blümmel *et al.* (1997). Gas yield (GP24) was calculated as the volume of gas (ml/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as: Gas yield (GY24) = Gas (ml/g DM)/DMD (g)

Short chain fatty acid concentrations (SCFA) were calculated according to Getachew *et al.* (2002) as: SCFA (mmol/200 mg DM) = 0.0222GP-0.00425, where, GP is the 24 h net gas production (mL/200 mg DM).

Microbial CP biomass production was calculated according to Blümmel *et al.* (1997) as : MCP (mg/g DM) = DMD (mg) – Gas (mL)×2.2 (mg/mL), where, 2.2 (mg/mL) is a stoichiometric factor which expresses mg of C, H and O required for production of SCFA gas associated with production of 1 mL of gas.

Statistical analysis

All data were analyzed as a Completely Randomize Design using the general linear procedure in PROC GLM of SAS (1996). The significant group differences were compared by Duncan's New Multiple Range Test and orthogonal polynomial contrast (Steel and Torrie, 1980)

Results and Discussion

The chemical composition

The chemical compositions of dietary treatments are shown in Table 1. Results of the proximate analysis showed the crude protein content in rice straw is 3.0%, 93.9% OM and 1.2% EE of dry matter basis. This results similarly reported by Napasirth *et al.* (2012) who reported the untreated rice straw contain 3.8% CP of dry matter basis. Moreover, forage fiber analysis for rice straw in this experiment are showed that the rice straw contained 80.7% NDF, 53.0 %ADF and 5.7 ADL. According to Togtokhbayar *et al.* (2015) who found that wheat straw contain more than 70% NDF on a dry matter (DM) basis, with less than 40% total digestive tract digestibility of the NDF, even under ideal feeding conditions (NRC, 2001).

Table 1 The chemical composition of dietary treatments

	DM, %	Chemical composition, % of DM basis						
		OM	Ash	CP	EE	NDF	ADF	ADL
Concentrate	96.3	93.4	6.7	11.0	1.1	33.2	11.4	3.6
Rice straw	93.8	93.9	6.1	3.0	1.2	80.7	53.0	5.7

DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, EE = Ether Extract
NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ADL = Acid Detergent Lignin

Gas production and gas characteristic

There were no effect (P>0.05) of EFE supplementation at doses of 0, 250, 500 and 750 mg/kg diets on the fermentation of the soluble fraction (a) (3.84, 2.95, 3.14 and 2.72 ml/0.2 gDM), the fermentation of the insoluble fraction (b) (50.4, 50.4, 49.7 and 51.1 ml/0.2 gDM), rates of gas production (c) (0.06, 0.057, 0.055 and 0.057 ml/h), potential of gas production (54.5, 53.4, 52.8 and 53.9 ml/0.2 gDM) and gas production at different incubation times (GP2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96) (Table 2, Fig. 1). These results agree with Salem *et al.,* 2015) who reported that treatment with

exogenous fibrolytic enzymes had no effect neither on parameters of GP nor on *in vitro* degradation and pattern of fermentation and no effect of dietary enzyme supplementation on ruminal fermentation parameters was also noted in other studies (Beauchemin *et al.*,1999).

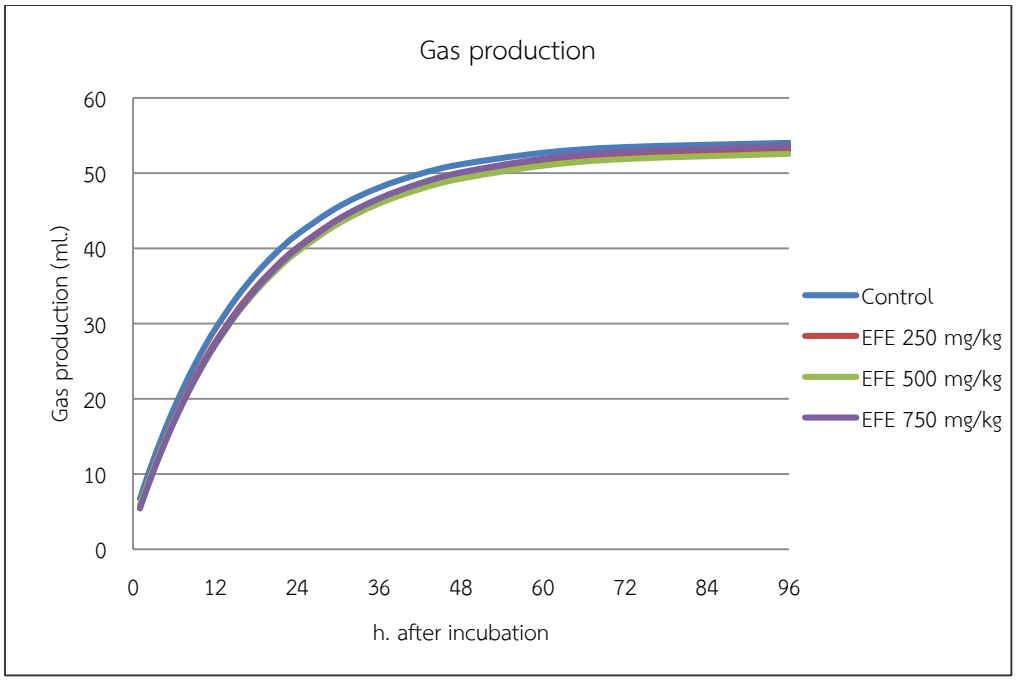

Fig. 1 Effect of exogenous fibrolytic enzymes (EFE) on gas accumulation of dietary treatments

However, GP6 ($P<0.05$) was cubically decrease ($P<0.01$) and GP4 was linearly decrease ($P<0.05$) with increasing level of EFE supplementation. Cumulative gas production volume at 4 and 6 h. after incubation was decreased by supplementing EFE, this result suggests that EFE supplementation was released more reducing sugars from forage (Colombatto *et al.,* 2003) and can promote a greater flow of carbon for volatile fatty acids and/or microbial protein synthesis (Mendoza *et al.*, 2014).

***In vitro* degradability and rumen metabolites**

The *in vitro* fermentation profiles are presented in Table 3. There were significantly different of EFE supplementation for *in vitro* dry matter digestibility (IVDMD, $P<0.01$), *in vitro* organic matter digestibility (IVOMD, $P<0.05$), metabolizable energy (ME, $P<0.05$), total digestible energy (TDN, $P<0.05$), the partitioning factor of incubation (PF, $P<00.5$), the gas yield (GY, $P<00.5$) and microbial crude protein production (MCP, $P<0.05$) at 24 h. after incubation, with no effect on ammonia-nitrogen (NH_3-N) and short chain fatty acid concentration (SCFA, $P>0.05$). Moreover, there were no effect ($P>0.05$) of treatments on all parameter measured at 48 h. after incubation.

Table 2 Effects ofexogenous fibrolytic enzymes supplementation on *in vitro* gas production of dietary treatments

	EFE (mg/kg)				SEM	P-value	Contrast		
	0	250	500	750			Linear	Quadratic	Cubic
Gas production characteristics									
a	3.84	2.95	3.14	2.72	0.65	0.49	0.19	0.65	0.48
b	50.4	50.4	49.7	51.1	0.91	0.59	0.63	0.35	0.39
c	0.06	0.057	0.055	0.057	0.00	0.31	0.17	0.19	0.84
Potential GP, (ml/0.2g)									
	54.5	53.4	52.8	53.9	1.15	0.65	0.59	0.26	0.81
Gas production, (ml/0.2g)									
GP2	8.34	7.27	7.41	7.39	0.54	0.48	0.27	0.33	0.57
GP4	14.3	12.7	13.5	11.9	0.65	0.09	0.02	0.44	0.51
GP6	19.5[a]	17.9[ab]	17.5[ab]	16.9[b]	0.07	0.01	0.43	0.64	<0.01
GP8	23.9	22.2	21.8	21.6	0.72	0.13	0.03	0.30	0.70
GP10	27.2	25.3	25.3	25.4	0.76	0.24	0.12	0.21	0.61
GP12	30.1	28.4	28.4	28.2	0.78	0.34	0.16	0.25	0.77
GP24	40.6	38.9	38.3	28.9	0.89	0.32	0.17	0.30	0.99
GP36	46.8	45.4	44.5	45.2	0.95	0.42	0.19	0.30	0.81
GP48	50.6	49.3	48.4	49.1	0.98	0.49	0.25	0.32	0.82
GP72	54.2	53.1	52.7	53.6	1.01	0.77	0.66	0.34	0.90
GP96	56.0	54.9	54.4	55.5	1.02	0.70	0.65	0.28	0.85

[ab] Means in the same row with different superscript differ (P<0.05)

a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction, d = the potential extent of gas production, EP = a + [bc/(k + c)] where k = 0.05 (Ørskov and McDonald, 1979)

These results confirmed with results from Newbold (1997) and Togtokhbayar *et al.* (2015) who noted that most enzymes function within a few hours of feeding before being degraded by the proteolytic activity of rumen microbes. These results suggest that the treatment with EFE stimulated the feed degradation only during the initial phases, but the effects were reduced as incubation time progressed.

The 500 mg/kg of EFE doses recorded as the highest IVDMD (cubic effect, P<0.01), IVOMD (cubic effect, P<0.01), ME (cubic effect, P<0.01), ammonia nitrogen (NH_3-N; cubic effect, P<0.05), TDN (cubic effect, P<0.01), PF (cubic effect, P<0.05) and MCP (cubic effect, P<0.01). These results agree with Llewellyn *et al.* (2010) who studied in wheat straw and showed that IVDMD enhanced by EFE compared to the control group. Similarly, Elwakeel *et al.* (2007) who pointed that there is evidence that exogenous enzymes increase microbial protein synthesis, an indicator that the bacterial population of the rumen is increased. Moreover, Alvarez *et al.* (2009) exposed enzymes tended to increase particle outflow rate from the rumen associated with a reduction in rumen liquid viscosity. However, increasing level of EFE resulted in cubic trend of IVDMD, IVOMD and ME. This result was established by Togtokhbayar *et al.* (2015) who showed that the optimal dose for the degradability of wheat straw was intermediate dosage compared with the low or high levels of enzyme addition. The more impact of moderate amounts of EFE than higher amounts approves with the reported by Beauchemin *et al.* (2000), in which larger amounts of enzyme preparations did not develop DM digestibility when compared with lower amounts of enzymes. Moreover, Colombatto *et al.* (2003) determined that the rate and extent of GP were increased by increasing the level of enzyme from 1 time to5 times, but levels of 10 times were ineffective and this observed was also supported by Lewis *et al.* (1999) and Nsereko *et al.* (2002) who have shown that it is possible to over-supplement. Increased dose of fibrolytic enzymes may have prohibited binding of enzymes to receptors of substrate, which reduced comparative attachment by fibrolytic microorganisms to fiber (Treacher and Hunt 1996). Beauchemin *et al.*(2003) also speculated that excessive enzymes supplemented to the feed may bind to fiber sites used by ruminal microorganism and make them unavailable, creating a barrier against microbial colonization.

Conclusions

The present study found that the sum of the gas production did not differ among treatments but increasing the supplementing of EFE in the diet decreased the gas accumulation at 4 and 6 h after incubation. In addition, the 500 mg/kg of EFE doses recorded the highest IVDMD, IVOMD, ME, NH_3-N, TDN, PF and MCP. Therefore, this study suggested that EFE supplementation at 500 mg/kg DM was the suitable level to enhance fiber degradation and improve energy density of TMR with 50% rice straw and 50% concentrate.

Table 3 Effects of exogenous fibrolytic enzymes supplementation on *in vitro* fermentation profile

	EFE (mg/kg)				SEM	P-value	Contrast		
	0	250	500	750			Linear	Quadratic	Cubic
In vitro dry matter digestibility, IVDMD (%)									
24 h	52.0^{b}	43.2^{c}	58.2^{a}	53.0^{b}	0.55	<0.01	0.01	0.07	<0.01
48 h	56.0	60.6	62.9	55.7	4.67	0.88	0.96	0.48	0.85
In vitro organic matter digestibility, IVOMD									
24 h	58.7^{ab}	53.2^{c}	62.3^{a}	57.9^{b}	0.73	0.01	0.21	0.62	<0.01
48 h	60.7	66.1	67.3	60.5	4.43	0.85	0.98	0.44	0.91
Metabolizable energy, ME (Mcal/kg DM)									
24 h	2.24^{ab}	2.03^{c}	2.38^{a}	2.21^{b}	0.03	0.01	0.19	0.62	<0.01
48 h	2.32	2.53	2.57	2.31	0.17	0.85	0.99	0.45	0.92
Ammonia nitrogen, NH_3-N									
24 h	13.9	13.0	15.1	14.2	0.34	0.13	0.22	1.00	0.04
48 h	23.0	20.6	20.6	20.9	0.89	0.52	0.32	0.33	0.72
Total digestible energy, TDN									
24 h	61.7^{ab}	55.9^{c}	65.5^{a}	60.8^{b}	0.76	0.01	0.21	0.62	<0.01
48 h	63.7	69.4	70.6	63.5	4.65	0.85	0.98	0.44	0.91
The partitioning factor, PF									
24 h	3.16^{b}	3.00^{b}	3.72^{a}	3.44^{ab}	0.08	0.03	0.03	0.64	0.02
48 h	2.49	2.85	2.98	2.6	0.22	0.76	0.76	0.36	0.87
Gas yield, GY24									
24 h	316.0^{a}	334.5^{a}	268.8^{b}	290.5^{ab}	7.91	0.04	0.04	0.89	0.02
48 h	419.5	350.8	335.2	385.2	36.74	0.75	0.65	0.38	0.96
Short chain fatty acid concentrations, SCFA									
24 h	3.36	2.95	3.20	3.15	0.06	0.10	0.37	0.09	0.06
48 h	4.61	4.35	4.32	4.37	0.06	0.24	0.13	0.18	0.77
Microbial CP biomass production, MCP									
24 h	146.2^{bc}	105.6^{c}	219.4^{a}	176.8^{ab}	8.54	0.01	0.01	0.93	<0.01
48 h	59.0	127.5	152.5	79.9	45.30	0.79	0.78	0.39	0.88

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