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Additives on *in vitro* ruminal fermentation characteristics of rice straw

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ABSTRACT - The objective of this study was to evaluate the effects of mineral and protein-energy (MPES), exogenous fibrolytic enzyme supplements (ES), combination of MPES + ES, and straw without supplement (WS) on digestibility, fermentation kinetic parameters, cumulative gas production, methane, CO_2 production, and volatile fatty acid concentration of rice straw of low and high nutritional value, estimated by *in vitro* techniques. The experimental design was randomized and factorial 2 × 4: two straws (low and high nutritional value) incubated with four supplements (MPES, ES, MPES + ES, and WS) and their interactions. Four experimental periods were used, totaling four replications per treatment over time. Data were analyzed by PROC MIXED of SAS. The *in vitro* dry matter and organic matter digestibilities of the rice straw with high nutritional value was improved by MPES, while the combination of MPES + ES supplements inhibited the digestibility of this straw. Dietary carbohydrate and nitrogen increased through MPES and MPES + ES supplements resulted in an increase in NH_3-N concentration and a decrease in CO_2 production due to the microbial mass formation. However, this increase was not enough to improve organic matter degradability parameters, cummulative gas production, gas production kinetics, and acetate:propionate ratio and reduce methane emissions. The straw with high nutritional value showed greater content of nitrogen fraction a, effective degradability, cummulative gas production, and methane and CO_2 productions comparing with low-nutritional value straw. The use of MPES and MPES + ES supplements can be used as strategy to mitigate CO_2 in ruminant production systems that use rice straw.

Key Words: digestibility, digestion kinetics, fatty acid, gas production, methane

Introduction

Rice is the second most cultivated cereal worldwide (FAO, 2016). Therefore, for each ton of rice grain harvested, one ton of straw remains in the field (Doyle et al., 1986). Despite the low nutritional value of rice straw due to its high silica content, low ruminal degradation of carbohydrates, and low nitrogen content, when stored in bales, it presents significant potential for strategic use in critical periods of food availability or in ruminant production systems with low nutrient requirements.

Technologies to increase the use of low-quality feeds, such as rice straw, consist in optimizing nutrient availability for the ruminal fermentation, ensuring no deficiency of nutrients to the microorganisms. Increased bacterial growth may result in increased extraction, through fermentation, of the roughage carbohydrate energy and, as a result, microbial cells synthesized in the rumen are available for amino acid digestion and absorption in the intestine (Leng, 1990).

This nutrient availability optimization in cattle fed rice straw may be achieved with mineral and protein-energy supplementation aiming to improve forage digestibility to maximize its intake (Barbosa et al., 2007), meeting animal requirements for maintenance and moderate weight gain (Lima, 2002). Furthermore, this supplementation increases the ruminal ammonia nitrogen concentration and meets the requirements of the ruminal microorganisms, allowing maximum fermentation rates (Fike et al., 1995).

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Additionally, the increase in the metabolizable protein availability and the increase in the absorbed protein:energy ratio reduced the metabolic heat production, promoting greater intake and raising the gain rates (Leng, 1990; Poppi and McLennan, 1995).

On the other hand, supplementation with feed of low nutritional value, with exogenous fibrolytic enzymes, aims to increase nutrient use and animal production efficiency (Nsereko et al., 2000; Beauchemin et al., 2003) and reduce the fecal output. These enzymes potentiate the degradation of fibrous polysaccharides togheter with the enzymes produced by the rumen microorganisms, stimulating total digestion and degradation rate, thus improving the digestibility of fibrous feeds (Newbold, 1997).

The hypothesis that the improvement of the rice straw *in vitro* fermentation process may be achieved by using additives was investigated. Therefore, the objective of this study was to evaluate, through *in vitro* techniques, the effects of mineral and protein-energy and exogenous fibrolytic enzyme supplements on digestibility, fermentation parameters and kinetics, maximum gas production, methane (CH_4) and carbon dioxide (CO_2) production, and volatile fatty acid concentration in rice straw.

Material and Methods

Animal care procedures throughout the study followed protocols approved by the Ethics Committee for Animal Use (ECAU) of the Universidade Federal do Rio Grande do Sul, number 18442/2010.

Two straws (low and high nutritional value) were incubated *in vitro* without supplementation (WS), with mineral and protein-energy supplement (MPES), with exogenous fibrolytic enzymes supplement (ES), and with the combination of the two supplements (MPES + ES) (Table 1). Four experimental periods were used, totaling four treatment replications. Duplicate bottles were also included in each run as blanks.

The mineral and energy-protein supplement used was commercially available and prepared in compliance with the nutritional standards of the NRC (1996) for beef cattle, whose daily intake recommendation is 50 g 100 kg^{-1} body weight, composed of non-protein nitrogen source, macro and micro minerals, cottonseed and soybean meal, and wheat bran. The enzyme supplement was a commercially available source of xylanase, whose daily intake recommendation is 15 g $head^{-1}$, consisting of corn distillers' dried grains with solubles, plant protein products, *Yucca schidigera* plant extract, and dried *Trichoderma longibrachiatum* fermentation extract (Alltech Inc.).

The straw rice intake of 100 g kg^{-1} of body weight was considered.

The *in vitro* digestibility was determined by the two-stage digestion technique proposed by Tilley and Terry (1963). Ruminal inoculum was collected from two fasting Texel sheep with an average weight of 60 kg adapted for 10 days to a diet based on alfalfa hay. Two hours after morning feed, rumen fluid and part of the rumen solid material were obtained to collect microorganisms adhered to the substrate. All collected material was homogenized in a blender at a ratio of 1:1 (solid:liquid portion) and filtered through four layers of gauze adding CO_2.

In vitro organic matter digestibility (IVOMD) was calculated by the difference between the incubated and undigested organic matter (OM) present in crucibles (Goering and Van Soest, 1970).

Table 1 - Chemical composition and *in vitro* organic matter digestibility (IVOMD) of rice straw and mineral and protein-energy (MPES) and enzyme (ES) supplements used in the experimental diets

Chemical composition (g kg^{-1} of dry matter)	High-nutritional value straw	Low-nutritional value straw	MPES[1]	ES
Dry matter[2]	873	915	927	915
Organic matter[2]	831	828	400	955
Crude protein[2]	44.7	37.4	398	240
Neutral detergent fiber[3]	732	781	196	317
Acid detergent fiber[4]	424	469	69.8	130
Cellulose[5]	393	435	45.3	90.7
Hemicellulose[5]	307	302	126	186
Lignin[5]	28.0	34.4	24.5	39.6
Silica[5]	95.9	126	-	-
IVOMD	529	424	653	765

[1] Each kg contains: 60 g calcium; 30 g phosphorus; 14 g sulfur; 74 g sodium; 0.21 g manganese; 1.3 g zinc; 0.06 g cobalt; 0.12 g copper; 0.02 g iodine; 0.009 g selenium; 0.28 g fluorine; 330 g non-protein nitrogen; and 220 g total digestible nutrients.
[2] Determined according to AOAC (1975).
[3] Not assayed with stable amylase expressed exclusive of residual ash acid.
[4] Expressed inclusive of residual ash.
[5] Determined according to Van Soest et al. (1991) but with a modification to determine silica, in which the residue was burned in a muffle furnace at 550 °C overnight.

In vitro cumulative gas production was obtained through the Theodorou et al. (1994) methodology modified by Mauricio et al. (1999), using a pressure transducer data logger (PDL 200 LANA/SCENE USP, Piracicaba/SP, Brazil) connected to a three-output valve. The first output was connected to the pressure transducer, the second to the needle (no. 22) to be inserted into the bottle stopper, and the third to a plastic syringe to measure the volume. Ruminal inoculum was obtained as described previously. The bottles were sealed with rubber stoppers and aluminum rings. In each experimental period, two bottles per treatment and per time were incubated, totaling 224 bottles plus the blank bottles (two blanks per incubation time, totaling 28 bottles). Four experimental periods were used, totaling four replications per treatment over time.

Pressure and volume of gas were measured at 0, 1, 3, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72, and 96 h post-incubation. Gas production was expressed in mL of gas produced per gram of organic matter incubated.

For degradation rate adjustment, gas production data were fitted using a bicompartimental model (Schofield et al., 1994): $V(t) = A / (1 + \exp \times (2 - 4 \times B \times (T - C))) - 1 + D / (1 + \exp (2 - 4 \times E \times (T - F))) - 1$, in which V (t) = cumulative gas production at time t (mL g^{-1} OM); A = maximal gas production of the rapid fermentation fraction (mL); B = fermentation rate of A (h); C = lag time of the fraction A (h); D = maximal gas production of the slow fermentation fraction (mL); E = fermentation rate of D (h); F = lag time of the fraction D (h); and T = incubation time (h). The model parameters were estimated by interactive Marquardt method inserted into the NLIN procedure of SAS (Statistical Analysis System, version 9.3).

The partition factor (PF) was determined according to Makkar (2004): FP = mg OM truly degraded/mL gases. For this calculation, we considered 36 h of incubation (time in which half of the maximal gas production was produced by treatments).

At 6, 12, 24, 48, 72, and 96 h, the fermentation was stopped and the pH measurements were performed immediately. Subsequently, the contents of the bottles were filtered through a sintered-glass crucible of coarse porosity (100 to 160 μm). Crucible containing residue from the filtration was oven-heated at 105 °C for 12 h, weighed, resulting in a moisture-free residue, and subsequently heated at 450 °C for 5 h. *In vitro* organic matter degradability was calculated by the difference between the incubated and undigested organic matter present in crucibles (Goering and Van Soest, 1970).

To study the ruminal degradability kinetics, the degradability obtained at different times was adjusted using the McDonald (1981) model: $Yt = a + b (1 - \exp - c (t - to))$, in which Yt = losses for degradation after t hours; a = immediately solubilized substrate; b = insoluble material, but potentially degradable; a + b = potential degradability; c = degradation rate of b; t = incubation time (h); and to = lag time. The effective degradability (ED) was calculated using the equation proposed by Ørskov and McDonald (1979): $ED = a + [(b \times c) / (c + k)] \exp (-(c + k) t)$, in which a, b, c, and t followed previous definitions and k = ruminal outflow rates of 0.02 or 0.05 h^{-1}.

At these same times, two aliquots of 5 mL of the filtrate were collected, one for volatile fatty acid and another for ammonia nitrogen (NH_3-N) determination. In the aliquots for NH_3-N determinations, 1 mL of 0.18 molar sulfuric acid was added to avoid nitrogen losses. Aliquots were frozen until analysis.

Volatile fatty acid concentrations - acetic (C2), propionic (C3), and butyric acids (C4) - were determined by high-performance liquid chromatography, in a chromatograph (Shimadzu model 14-B) equipped with UV detector, pre-column and column (Aminex HPX-87H, BioRad®). Sulfuric acid was used as eluent at 0.01 molar concentration, at a 0.6 mL min^{-1} flow rate and 50 °C operating temperature. The detection wavelength was set at 210 nm. Volatile fatty acid concentrations were calculated from the calibration curves using standards (Sigma®, analytical grade) at 0.1 to 2.5 g L^{-1} concentrations.

Ammonia nitrogen concentrations were determined by magnesium oxide distillation according to AOAC (1995).

The volume of gas produced during the intervals of 12, 24, 36, 48, 72, and 96 h of incubation was collected, measured, and stored in 20-mL vacutainer tubes without additive for the gas analysis.

Methane and CO_2 gases were analyzed by gas chromatography (Shimadzu® "greenhouse" model) equipped with three packed columns operating at 70 °C. Nitrogen as a carrier gas (25 mL min^{-1}), injector (250 °C) with direct sampling of 1 mL, and electron capture detector with Ni_{63} at 325 °C were used.

Peak gas areas were determined automatically by integration. Methane volume produced at time x was calculated in accordance with Tavendale et al. (2005): CH_4 production (mL g^{-1} dry matter (DM)) at time $x = (\% CH_4 (x) - \% CH_4 (x - 1)) \times 40/100 + CH_4\% (x) \times GP / 100$, in which x time = 12, 24, 36, 48, 72, and 96 fermentation h; x – 1 = previous time; 40 = head space in the fermentation bottle in mL; GP = volume of gas produced in mL. This calculation resulted in the volume of CH_4 gas produced between each time interval. The sum of these volumes resulted in the

accumulated volume of CH_4 for 96 h. The same formula was used to calculate CO_2 production.

Digestibility, degradation parameters, effective degradation of organic matter, maximum gas production from the rapidly and slowly degradable fractions and their respective degradation rates, time of colonization, and partition factor data were analyzed using the PROC MIXED of SAS. The following statistical model was used:

$$Yijkl = \mu + \alpha i + \beta j + \alpha\beta ij + \gamma k + eijkl,$$

in which Yijkl = dependent variables; μ = overall mean of the observations; αi = fixed effect of the straw (i = 1, 2); βj = fixed effect of the supplement (j = 1, 2, 3, 4); αβij = straw × supplement interaction effect (i = 1, 2, and j = 1, 2, 3, 4); γk = ramdom effect of the period (k = 1, 2, 3, 4); and eijkl = random residual experimental error.

Gas production, pH, NH_3, CH_4, CO_2, and VFA data were analyzed as repeated measures over time using the same procedure. The following statistical model was used:

$$Yijklm,1 = \mu + \alpha i + \beta j + \alpha\beta ij + \varepsilon ij + \tau k + \alpha\tau ik + \beta\tau jk + \alpha\beta\tau ijk + \gamma l + eijklm,$$

in which Yijklm,1 = dependent variables; μ = overall mean of the observations; αi = fixed effect of straw (i = 1, 2); βj = fixed effect of the supplement (j = 1, 2, 3, 4); αβij = straw × supplement interaction effect (i = 1, 2, and j = 1, 2, 3, 4); εij = random residual experimental error; τk = fixed effect of the time ((k = 6, 12...96), or k = 12, 24…96)); ατik = straw × time interaction effect; βτjk = supplement × time interaction effect; αβτijk = straw × supplement × time interaction effect; γl = ramdom effect of the period (k = 1, 2, 3, 4); and eijklm = experimental error associated with the observation Yijklm,l level.

Using Akaike information criterion, the CS structure (symetry compound) was regarded as the best model for the residual covariance structure.

Results

There was a significant interaction between the supplement and rice straw nutritional value for *in vitro* dry matter and organic matter digestibilities (Table 2). The mineral and protein-energy supplement improved the *in vitro* dry matter and organic matter digestibility of the rice straw with high nutritional value, while the rice straw with low nutritional value without supplementation showed lower values for *in vitro* dry matter and organic matter digestibility.

Organic matter degradation parameters were influenced only by the rice straw nutritional value (Table 3). Straw with high nutritional value had greater content of readily soluble fraction of OM (a) ($P<0.05$) compared with the straw with low nutritional value, 188 and 162 g kg^{-1} OM, respectively.

Averages of insoluble fraction, but potentially degradable (b), degradation rate of the insoluble fraction, but potentially degradable (c), and lag time of OM were 651 g kg^{-1} OM, 0.0017 h^{-1}, and 9.56 h, respectively (Table 3), without influence of the straw nutritional value.

Table 2 - Interaction effect between the supplement and the rice straw nutritional value on the *in vitro* dry matter (IVDMD) and *in vitro* organic matter (IVOMD) digestibility

Suplement	Rice straw nutritional value		Mean	SEM
	High	Low		
IVDMD (g kg^{-1} fresh material)				
Without supplement	473Ba	392Bb	432	0.2190
Mineral and protein-energy supplement (MPES)	519Aa	423Ab	472	0.2190
Exogenous fibrolytic enzyme supplement (ES)	467Ba	435Ab	451	0.2190
MPES + ES	475Ba	422Ab	448	0.2190
Mean	484	417		
SEM	0.1548	0.1548		
Significance (P =)				
Nutritional value × suplement	<0.0001			
IVOMD (g kg^{-1} DM)				
Without supplement	568Ba	495Bb	532	0.2633
MPES	604Aa	515Ab	559	0.2633
ES	561Ba	529Ab	545	0.2633
MPES + ES	551Ba	519Ab	535	0.2633
Mean	571	515		
SEM	0.1862	0.1862		
Significance (P =)				
Nutritional value × suplement	<0.0001			

DM - dry matter; SEM - standard error of the mean.
Different uppercase letters in the column and different lowercase letters in the row differ statistically ($P<0.05$) by Tukey test.

Effective degradability obtained for the solid fraction passage rates (k = 0.02 and 0.05 h^{-1}) were influenced by the straw nutritional value (P<0.05) (Table 3).

There was an interaction effect between the straw nutritional value and the incubation time on the *in vitro* cumulative gas production (Table 4). From 18 h of incubation, the cumulative gas production of high-nutritional value straw was greater than for the low-nutritional value straw and at the end of the 96 h of incubation, this production was 200.22 and 186.13 mL g^{-1} OM, respectively, showing better fermentation for the high-nutritional value straw.

Maximum gas production of the rapidly degradable fraction of organic matter (A) was influenced by the straw nutritional value. High-nutritional value straw produced 113.98 mL, while the low value produced 75.61 mL of gases related to fraction A. However, the maximum gas production of the slowly degradable fraction of organic matter (D) was influenced by straw nutritional value and by the supplement. Low- and high-nutritional value straws produced 113.43 and 89.27 mL, respectively (P<0.05). Supplements contributed to the reduction in the maximum gas production related to fraction D compared with the treatment without supplement (Table 5).

Table 3 - Effect of the rice straw nutritional value and the supplement on the organic matter degradation parameters (a, b, c, and lag time) and organic matter effective degradability (g kg^{-1} OM) mesuared at outflow rate k = 0.02 and 0.05 h^{-1}

Parameter	a (g kg^{-1} OM)	b (g kg^{-1} OM)	c (h)	lag time (h)	ED 0.02	ED 0.05
Straw nutritional value						
High	188A	656	0.017	9.41	442A	297A
Low	168B	645	0.017	9.71	408B	266B
SEM	0.53	2.32	0.0012	0.68	0.74	0.65
Suplement						
Without supplement	183	661	0.016	10.25	424	277
Mineral and protein-energy supplement (MPES)	193	675	0.016	9.83	425	283
Exogenous fibrolytic enzyme supplement (ES)	187	652	0.017	9.30	420	276
MPES + ES	192	617	0.019	8.86	432	289
SEM	0.74	3.28	0.0017	0.96	1.05	0.92
Significance (P =)						
Nutrition value	<0.0001	0.5401	0.7170	0.4988	<0.0001	<0.0001
Suplement	0.1572	0.1300	0.1445	0.1536	0.4108	0.1849
Nutritional value × suplement	0.3115	0.6349	0.2517	0.5617	0.4301	0.3154

OM - organic matter; ED - effective degradability; SEM - standard error of the mean.
Different uppercase letters in the column differ statistically (P<0.05) by Tukey test.

Table 4 - Effect of interaction between the rice straw nutritional value and the incubation time on the *in vitro* cumulative gas production (mL g^{-1} OM)

Incubation time (hours)	Rice straw nutritional value		Mean	SEM
	High	Low		
1	0.03K	0I	0.01	1.30
3	0.92K	0.84I	0.87	1.30
6	5.45JK	2.12I	3.78	1.30
9	10.57IJ	4.58I	7.66	1.30
12	15.62I	7.12I	11.37	1.30
18	30.82Ha	18.63Hb	24.72	1.30
24	50.82Ga	36.26Gb	43.54	1.30
30	78.74Fa	60.36Fb	69.55	1.30
36	103.69Ea	83.38Eb	93.53	1.30
48	134.97Da	114.84Db	124.90	1.30
60	161.71Ca	144.86Cb	153.28	1.30
72	179.93Ba	165.40Bb	172.66	1.30
96	200.22Aa	186.13Ab	193.18	1.30
Mean	69.54	58.89		
SEM	0.49	0.49		
Significance (P =)				
Nutritional value × incubation time	<0.0001			

OM - organic matter; SEM - standard error of the mean.
Different uppercase letters in the column and different lowercase letters in the row differ statistically (P<0.05) by Tukey test.

The degradation rate of slowly degradable fraction (E) was greater for the low-nutritional value straw than the high nutritional value, 0.026 and 0.022 h^{-1}, respectively (P<0.05). The lag times of rapidly (C, h) and slowly degradable (F, h) fractions were greater for the high-nutritional value straw than for the lower value (P>0.05). The partition factor, was not affected by treatments (P>0.05), indicating that the fermentation efficiency was not affected by supplementation (Table 5).

The pH decreased, while the NH_3-N concentration increased with the increase in incubation time (Table 6). The NH_3-N concentrations were also influenced by the straw

Table 5 - Effect of the rice straw nutritional value and the supplement on the maximum gas production of organic matter of the rapidly (A, mL) and slowly (D, mL) degradable fractions and their respective degradation rates (B and E, h), lag time (C and F, h), and partition factor (mg OM/mL gases, 36 h of incubation)

Parameter	A (mL)	B (h)	C (h)	D (mL)	E (h)	F (h)	PF (mg mL^{-1})
Straw nutritional value							
High	113.98A	0.044	19.74A	89.27B	0.022B	36.34A	2.68
Low	75.61B	0.038	13.04B	113.43A	0.026A	30.60B	2.61
SEM	5.08	0.33	1.60	5.56	0.15	2.12	0.12
Suplement							
Without supplement	94.05	0.043	14.92	110.90A	0.022	34.91	2.37
Mineral and protein-energy supplement (MPES)	100.01	0.041	14.66	88.08B	0.022	32.17	2.88
Exogenous fibrolytic enzyme supplement (ES)	93.17	0.041	18.39	99.26AB	0.024	33.40	2.73
MPES + ES	91.96	0.041	17.58	107.15AB	0.024	33.41	2.60
SEM	7.19	0.47	2.73	7.86	0.22	3.00	0.17
Significance (P =)							
Nutrition value	<0.0001	0.0987	0.0004	0.0003	0.0242	0.0134	0.6844
Suplement	0.6895	0.9635	0.2803	0.0408	0.6238	0.8402	0.2867
Nutritional value × suplement	0.4071	0.8900	0.5968	0.6769	0.2910	0.9407	0.2298

PF - partition factor; OM - organic matter; SEM - standard error of the mean.
Different uppercase letters in the column differ statistically (P<0.05) by Tukey test.

Table 6 - Effect of the rice straw nutritional value, the supplement, and the incubation time on the pH, amonical nitrogen (NH_3-N), and carbon dioxide (CO_2) values

Parameter	pH	NH_3-N (mg dL^{-1})	Incubation time (h)	CO_2 (mL g^{-1} DM)
Straw nutritional value				
High	7.10	14.05B		19.03A
Low	7.08	14.38A		16.85B
SEM	0.028	0.11		0.1896
Suplement				
Without supplement	7.00	13.35B		18.74A
Mineral and protein-energy supplement (MPES)	7.12	14.79A		17.40B
Exogenous fibrolytic enzyme supplement (ES)	7.11	13.78B		18.07AB
MPES + ES	7.12	14.93A		17.57B
SEM	0.040	0.15		0.2670
Incubation time (h)				
6	7.19A	11.87E	12	19.63A
12	7.09AB	12.54DE	24	19.80A
24	7.12AB	13.22D	36	19.27A
48	7.10AB	14.30C	48	17.08B
72	7.05AB	15.84B	72	16.00B
96	6.94B	17.51A	96	15.88B
SEM	0.050	0.19		0.4549
Significance (P =)				
Nutritional value	0.6949	0.0399		<0.0001
Suplement	0.1095	<0.0001		0.0020
Incubation time	0.0042	<0.0001		<0.0001
Nutritional value × suplement	0.8219	0.9383		0.4105
Nitritional value × incubation time	0.3318	0.1139		0.5586
Suplement × incubation time	0.7613	0.5552		1.000
Nutritional value × supplement × incubation time	0.4054	0.8061		1.000

DM - dry matter; SEM - standard error of the mean.
Different uppercase letters in the column differ statistically (P<0.05) by Tukey test.

nutritional value (P<0.05) and the supplement (P<0.05). Low- and high-nutritional value straw showed NH_3-N concentrations of 14.05 and 14.38 mg dL^{-1}, respectively. The MPES + ES and MPES supplements showed the highest concentrations of NH_3-N, 14.93 and 14.79 mg dL^{-1}, respectively, differing from the supplements ES and WS, whose NH_3-N concentrations were 13.78 and 13.35 mg dL^{-1}, respectively (Table 6).

The volume of CO_2 produced (mL g^{-1} DM) was related to the straw nutritional value, the supplement, and the incubation time (Table 6). Low-nutritional value straw produced 16.85 mL g^{-1} DM, while the high value produced 19.03 mL g^{-1} DM of CO_2. The supplements MPES and MPES + ES produced lower volumes of CO_2 (17.40 and 17.57 mL g^{-1} DM) compared with supplements WS and ES (18.74; 18.07 mL g^{-1} DM), being important for mitigating CO_2. As the incubation time increased from 12 to 96 h, the CO_2 production decreased from 19.63 to 15.88 mL g^{-1} DM, respectively.

There was an interaction between the straw nutritional value and the incubation time on *in vitro* CH_4 production (Table 7). In the first 12 h of fermentation, CH_4 production was similar between straws. Starting from 12 h to the end of the incubation period, there was a linear increase in the CH_4 volumes for both straws; however, greater CH_4 volume was produced by high-nutritional value straw compared with the low-nutritional value straw. Nevertheless, at the end of the 96-h incubation, the CH_4 production rate in the total gas volume was 0.15 for both straws.

Volatile fatty acid concentration and the acetate: propionate ratio were influenced by the interaction between the straw nutritional value and the incubation time (Table 8). The greater acetic acid concentration and the lowest propionic acid concentration were observed for the low-nutritional value straw with 6 h of incubation (71.80 and 21.03 mol 100 mol^{-1} total volatile fatty acid, respectively). Thus, the greater acetate:propionate ratio was also observed for this straw (3.60 mol 100 mol^{-1} total volatile fatty acid) (Table 8). However, the greater concentration of butyric acid was observed for the high-nutritional value straw with 6 h of incubation (7.61 mol 100 mol^{-1} total volatile fatty acid).

Discussion

In this study, the highest IVDMD and IVOMD were observed for the straw with less lignification and silicified cell wall (high nutritional value) when supplemented with mineral and protein-energy supplement. For the same straw, mineral and protein-energy supplement inhibited the effect of the exogenous fibrolitic enzyme, since the combination of these supplements resulted in a lower *in vitro* digestibility in relation to other supplements. These results suggest that the improvement of IVDMD and IVOMD depends on the forage chemical characteristics and the supplement used, corroborating with Morgavi et al. (2000), who stated that more detailed knowledge of the interaction between the supplement with the forage, the host, and the rumen microorganisms is necessary for the correct application of this technology. Previous research had also identified variation in rice straw digestibility (Vadiveloo, 1992; Vadiveloo, 1995) and improvement of forage *in vitro* digestibility with the use of mineral and protein-energy supplement (Barbosa et al., 2007) and exogenous fibrolytic enzyme (Beauchemin et al., 2003; Bassiouni et al., 2011).

With the use of supplements, an improvement in the parameters of *in vitro* organic matter ruminal degradability

Table 7 - Effect of the interaction between the rice straw nutritional value and the incubation time on the *in vitro* cumulative methane production (mL g^{-1} DM)

Incubation time (hours)	Rice straw nutritional value		Mean	SEM
	High	Low		
12	1.16E	0.75F	0.96	0.53
24	7.97Da	5.64Eb	6.80	0.53
36	14.97Ca	11.66Db	13.32	0.53
48	22.32Ba	18.46Cb	20.39	0.53
72	27.43Aa	23.18Bb	25.30	0.53
96	29.39Aa	27.03Ab	28.31	0.53
Mean	17.21	14.45		
SEM	0.45	0.45		
Significance (P =)				
Nutritional value × incubation time	0.0017			

DM - dry matter; SEM - standard error of the mean.
Different uppercase letters in the column and different lowercase letters in the row differ statistically (P<0.05) by Tukey test.

of rice straw was expected. However, the supplements did not help carbohydrate release and did not provide enough nitrogen to improve these parameters, probably due to the limiting nitrogen content in the incubated straws.

Gas production is an indirect measure of substrate degradation, mainly of carbohydrates (Menke, 1979). In the current research, there was interaction between the incubation time and the nutritional value of rice straw on the *in vitro* cumulative gas production. At 9 and 12 h of incubation, there was an increase in gas production due to accumulation of indirect gas products of reaction between the buffer and the propionic acid generated from the fermentation of rapidly degradable carbohydrates and the indirect gas that starts to be produced from the structural carbohydrate degradation. According to Chai et al. (2004), the gases produced in the first 3 h of incubation correspond to the soluble components of the fermentation. To the extent that the incubation time increased, the volume of gas produced was increased by the effect of the structural carbohydrate fermentation of the substrate (Theodorou

Table 8 - Effect of interaction between the rice straw nutritional value and the incubation time on the volatile fatty acid concentration (mol 100 mol^{-1} total volatile fatty acid)

Incubation time (hours)	Rice straw nutritional value		Mean	SEM
	High	Low		
Acetate				
6	67.58Ab	71.80Aa	69.69	0.54
12	68.55A	69.86AB	69.21	0.54
24	66.94A	67.85B	67.40	0.54
48	68.19A	67.73B	67.96	0.54
72	68.34A	66.84B	67.59	0.54
96	69.02A	68.71AB	68.86	0.54
Mean	68.10	68.80		
SEM	0.31	0.31		
Significance (P =)				
Nutritional value × incubation time	0.0057			
Propionate				
6	24.79Ba	21.03Db	22.91	0.44
12	25.73AB	23.20CD	24.46	0.44
24	27.15AB	25.96AB	26.55	0.44
48	27.81A	28.89A	28.35	0.44
72	26.51AB	28.17AB	27.34	0.44
96	25.79AB	25.97AB	25.88	0.44
Mean	26.30	25.53		
SEM	0.25	0.25		
Significance (P =)				
Nutritional value × incubation time	0.0057			
Butirate				
6	7.61Aa	7.16Ab	7.39	0.47
12	5.71AB	6.93A	6.32	0.47
24	5.90AB	6.18A	6.04	0.47
48	3.98B	3.37B	3.68	0.47
72	5.13B	4.98AB	5.05	0.47
96	5.18B	5.31AB	5.24	0.47
Mean	5.58	5.56		
SEM	0.19	0.19		
Significance (P =)				
Nutritional value × incubation time	<0.0001			
Acetate:propionate ratio				
6	3.12Ab	3.60Aa	3.36	0.31
12	2.71AB	3.08B	2.90	0.31
24	2.48B	2.67BC	2.58	0.31
48	2.47B	2.37C	2.41	0.31
72	2.60B	2.40C	2.50	0.31
96	2.73AB	2.68BC	2.70	0.31
Mean	2.68	2.80		
SEM	0.30	0.30		
Significance (P =)				
Nutritional value × incubation time	0.0103			

SEM - standard error of the mean.
Different uppercase letters in the column and different lowercase letters in the row differ statistically (P<0.05) by Tukey test.

et al., 1994) and at the end of 96 h of incubation, greater cumulative gas production was observed for high-nutritional value straw due to greater organic matter degradadability in this straw, corroborating with Menke et al. (1979). However, the fermentation pattern was similar between straws and gas production curves corresponded to the fermentation pattern to a substrate with forage predominance, in which, initially, sugars are fermented and later, the structural components (Getachew et al., 2005).

Supplementation with MPES and MPES + ES did not affect the *in vitro* cumulative gas production. Liu and Ørskov (2000) reported that treatment of rice straw with several levels of cellulase did not affect the cumulative gas production over 24 h of incubation. However, Eun et al. (2006) treated Akibali rice straw with six different enzyme products (1.25 mg g^{-1} DM) and found that only two (product composed of cellulase and hemicellulase and product containing protease) increased cumulative gas production over 24 h of incubation compared with untreated rice straw. Therefore, the inconsistencies in the responses with enzyme supplements were probably related to supplement characteristics, including the enzyme activities in the conditions of rumen (temperature and pH), as well as the substrate composition (Yang et al., 2011).

The partition factor is an indicator of fermentation efficiency; thus, high value of partition factor indicates a greater incorporation of degraded organic matter in microbial mass, thereby increasing the microbial synthesis efficiency. The greater the partition factor value, the greater the forage dry matter intake (Makkar, 2004) and lower the CH_4 production in ruminants (Blümmel et al., 1999). According to Makkar (2004), partition factor may vary from 2.74 to 4.41 mg of degraded OM mL^{-1} of gases produced. In the current research, the partition factor values ranged from 2.37 to 2.88 and were not influenced by the straw nutritional value or the supplement.

The pH is an important variable to indicate the rumen status (Gunun et al., 2013), since it regulates the affinity of microorganisms with the substrate. Thus, values near neutral pH improve the bacteria adhesion to the fiber (Allen and Mertens, 1988). In the current study, the pH ranged from 6.94 to 7.19. These values are considered optimal for the normal rumen fermentation, for the synthesis of volatile fatty acid, and microbial protein (Wanapat and Pimpa, 1999; Anantasook et al., 2012), and are also within the range of 6.2 to 7.2, considered appropriate for optimal microbial activity (Van Soest, 1994), as expected for diets based on forage.

In vitro NH_3-N concentration works as an indicator of protein degradability because there is no nitrogen absorption or recycling, as in the *in vivo* rumen environment (Detmann et al., 2011). The average NH_3-N concentration of all treatments was 14.21 mL dL^{-1} and was within the optimum ruminal NH_3-N range of 12 and 17 mL dL^{-1} for optimal fermentation and rumen microbial growth (Anantasook and Wanapat, 2012; Lunsin et al., 2012). As expected, MPES and MPES + ES supplements increased the dietary levels of carbohydrate and nitrogen, resulting in an increase in NH_3-N concentration levels and decrease in CO_2 production due to the microbial mass formation.

Within the first 12 h after incubation, there was lower CH_4 production for both straws evaluated, as this period includes the lag-time phase, in which there is no methanogenesis until the sites available for microbial attachment are saturated and these synthesize its structures and enzymes (Franco et al., 2013). The linear increase in CH_4 volume for high and low straw nutritional value, from 12 h until the end of the incubation period, was associated with the slowly digestible fraction fermentation and, consequently, with acetic and butyric acid production (Getachew et al., 2005; Lee et al., 2011). The production of CH_4 at 96 h of incubation was greater for high-nutritional value straw compared with low value straw, possibly due to better digestibility of the former, corroborating with Kurihara et al. (1995), who observed that CH_4 production in cows fed forage with low digestibility was lower than in cows fed high forage digestibility. However, this disagrees with other studies that observed that CH_4 production tends to decrease with increasing protein concentration and tends to increase with increasing fiber content of the feed (Johnson and Johnson, 1995; Getachew et al., 2005). Another possibility may be related to the fiber degradability, since forage with greater content of effectively degraded fiber promotes greater CH_4 production (Demarchi et al., 2003).

Due to the lack of supplement effect on the degradation parameters, cumulative gas production, gas production kinetics, and CH_4 production, volatile fatty acid concentrations were measured to further explore any potential effect of the supplements on the rumen fermentation. However, the supplements did not affect the volatile fatty acid concentrations, but these concentrations were influenced by the interaction between the straw nutritional value and the incubation time. High levels of volatile fatty acids observed at the beginning of the fermentation can be explained by rumen fluid being obtained from animals fed a diet based on alfalfa hay. The dominance of the acetic acid concentration observed in the current study shows that when the diet had high forage content, ruminal fermentation occurred preferentially in

this way and was associated with high CH_4 production, corroborating with Nussio et al. (2011).

Acetic:propionic acid ratio is an important point in rumen methanogenesis, since greater energy losses in the CH_4 form is related to the greater acetic:propionic acid ratio (Johnson and Johnson, 1995). Also, as the propionic acid is the most important fatty acid precursor of the glucose synthesis (Nagajara et al., 1997), a low acetic:propionic acid ratio reflects an improvement of the food nutritional value. In the current research, there was no influence of the supplement on the acetic:propionic acid ratio and on improvement of the straw nutritional value. The results did not differ from results of Eun et al. (2006), who observed reduction in theacetic:propionic acid ratio with EX and PROT enzymatic treatment of rice straw, suggesting that microbial interactions lead to decreased acetate and increased propionate formation from the products of cellulose and hemicellulose hydrolyses when certain types of exogenous enzymes were added to rice straw.

Conclusions

The use of mineral and protein-energy supplement and mineral and protein-energy + exogenous fibrolytic enzymes supplements can be used as strategy to mitigate carbon dioxide in ruminant production systems that use rice straws.

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References

Allen, M. S. and Mertens, D. R. 1988. Evaluating constraints on fiber digestion by rumen microbes. Symposium: rumen productivity. The Journal of Nutrition 118:261-270.

Anantasook, N. and Wanapat, M. 2012. Influence of rain tree pod meal supplementation on rice straw based diets using *in vitro* gas fermentation technique. Asian-Australasian Journal of Animal Science 25:325-334.

AOAC - Association of Official Analytical Chemistry. 1975. Official methods of analysis. 12th ed. AOAC International, Washington, DC.

AOAC - Association of Official Analytical Chemists. 1995. Official methods of analysis. 16th ed. AOAC, Washington, DC.

Bassiouni, M. I.; Gaafar, H. M. A.; Saleh, M. S.; Mohi El-Din, A. M. A. and Elshora, M. A. H. 2011. Evaluation of rations supplemented with fibrolytic enzyme on dairy cows performance. In situ ruminal degradability of different feedstuffs. Livestok Research for Rural Development. Available at: <http://www.lrrd.org/lrrd23/4/bass23081.htm> Accessed on: Nov. 24, 2015.

Barbosa, F. A.; Graça, D. S.; Maffei, W. E.; Silva Júnior, F. V. and Souza, G. M. 2007. Desempenho e consumo de matéria seca de bovinos sob suplementação protéico-energética, durante a época de transição água-seca. Arquivo Brasileiro de Medicina Veteterinária e Zootecnia 59:160-167.

Beauchemin, K. A.; Colombatto, D.; Morgavi, D. P. and Yang, W. Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. Journal of Animal Science 81:37-47.

Blümmel, M.; Mgomezulu, R.; Chen, X. B.; Makkar, H. P. S.; Becker, K. and Ørskov, E. R. 1999. The modification of an in vitro gas production test to detect roughage related differences in in vivo microbial protein synthesis as estimated by the excretion of purine derivates. Journal of Agricultural Science 133:335-340.

Chai, W. Z.; Gelder, A. H. and Cone, J. W. 2004. Relationship between gas production and starch degradation in feed samples. Animal Feed Science and Technology 114:195-204.

Demarchi, J. J. A. A.; Manella, M. Q. A.; Lourenço, J.; Alleoni, G. F.; Frigueto, R. S.; Primavesi, O. and Lima, M. A. 2003. Daily methane emission at different seasons of the year by Nelore cattle in Brazil grazing Brachiaria brizantha cv. Marandu: preliminary results. p.19. In: Proceedings of the 9th World Conference on Animal Production. SBZ, WAAP, ALPA, UFRGS, Porto Alegre.

Detmann, E.; Queiroz, A. C.; Zorzi, K. H.; Mantovani, C.; Bayão, G. F. V. and Gomes M. P. C. 2011. Degradação in vitro da fibra em detergente neutro de forragem tropical de baixa qualidade em função da suplementação com proteína verdadeira e/ou nitrogênio não-protéico. Revista Brasileira de Zootecnia 40:1272-1279.

Doyle, P. T.; Devendra, C. and Pearce, G. R. 1986. Rice straw as a feed for ruminants. 1th ed. International Development Program of Australian Universites and Coleges Limited, Canberra, AU.

Eun, J.-S.; Beauchemin, K. A.; Hong, S.-H. and Bauer, M. W. 2006. Exogenous enzymes added to untreated or ammoniated rice straw: Effects on in vitro fermentation characteristics and degradability. Animal Feed Science and Technology 131:86-101.

FAO - Food and Agriculture Organization of the United Nations. 2016. Rice market monitor. Available at: <http://www.fao.org/economic/est/publications/rice-publications/rice-market-monitor-rmm/en/ />. Accessed on: Oct. 29, 2016.

Fike, G. D.; Simmis, D. D.; Cochran, R. C.; Vanzant, E. S.; Kutil, G. L. and Brandt Jr, R. T. 1995. Protein supplementation of ammoniated wheat straw: effect on performance and forage utilization of beef cattle. Journal of Animal Science 73:1595-1601.

Franco, A. L. C.; Mizubuti, I. Y.; Azevêdo, J. A. G.; Ribeiro, E. L. A.; Pereira, E. S. Peixoto, E. L. T.; Ferreira, D. M. F. and Andrade Neto, A. Q. 2013. Fermentação ruminal e produção de metano *in vitro* de dietas contendo torta de algodão. Semina: Ciências Agrárias 34:1955-1966.

Getachew, G.; DePeters, E. J.; Robinson, P. H. and Fadel, J. G. 2005. Use of an in vitro rumen gas production technique to evaluate microbial fermentation of ruminant feeds and its impact on fermentation products. Animal Feed Science and Technology 123-124:547-559.

Goering, H. K. and Van Soest, P. J. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). Agriculture Handbook No. 379. ARS-USDA, Washington, D.C., USA.

Gunun, P.; Wanapat, M. and Anantasook, N. 2013. Effects of physical form and urea treatment of rice straw on runen fermentation, microbial protein synthesis and nutrient digestibility in dairy steers. Asian-Australasian Journal of Animal Science 26:1689-1697.

Johnson, K. A. and Johnson, D. E. 1995. Methane emissions from cattle. Journal of Animal Science 73:2483-2492.

Kurihara, M.; Kume, S.; Aii, T.; Takahashi, S.; Shibata, M. and Nishida, T. 1995. Feeding method for dairy cattle to cope with global warming. Technical assessment based on energy metabolism. p.21-107. In: The 29th bulletin of the Kyushu National Agricultural Experiment Station. Japan.

Lee, S. Y.; Lee, S. M.; Choa, Y. B.; Kam, D. K.; Lee, S. C.; Kim, C. H. and Seo, S. 2011. Glycerol as a feed supplement for ruminants: In vitro fermentation characteristics and methane production. Animal Feed Science and Technololgy 166-167:269-274.

Leng, R. A. 1990. Factors affecting the utilization of "poor quality" forages by ruminants particularly under tropical conditions. Nutrition Research Reviews 3:277-303.

Lima, J. O. A. A. 2002. Mistura múltipla para bovinos em pastejo na região dos tabuleiros costeiros. EMBRAPA dos Tabuleiros Costeiros, Aracajú.

Liu, J. X. and Ørskov, E. R. 2000. Cellulase treatment of untreated and steam pre treated rice straw-effect on in vitro fermentation characteristics. Animal Feed Science and Technology 88:189-200.

Lunsin, R.; Wanapat, M. and Rowlinson, P. 2012. Effect of cassava hay and rice bran oil supplementation on rumen fermentation, milk yield and milk composition in lactating dairy cows. Asian-Australasian Journal of Animal Science 25:1364-1373.

Makkar, H. P. S. 2004. Recent advances in the in vitro gas method for evaluation of nutritional quality of feed resources. p.55-88. In: Assessing quality and safety of the animal feeds. FAO Animal Production and Health Series Paper 160. FAO, Roma.

Mauricio, R. M.; Mould, F. L.; Dhanoa, M. S.; Owen, E.; Channa, K. S. and Theodorou, M. K. 1999. A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. Animal Feed Science and Technology 79:321-330.

McDonald, I. 1981. A revised model for the estimation of protein degradability in the rumen. Journal of Agricultural Science 96:251-252.

Menke, K. H.; Raab, L.; Salewski, A.; Steingass, H.; Fritz, D. and Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. Journal of Agricultural Science 93:217-222.

Morgavi, D. P.; Beauchemin, K. A.; Nsereko, V. L.; Rode, L. M.; Iwaasa, A. D.; Yang, W. Z.; McAllister, T. A. and Wang, Y. 2000. Synergy between ruminal fibrolytic enzymes and enzymes from Trichoderma longibrachiatum. Journal of Dairy Science 83:1310-1321.

Nagajara, T. G.; Newbold, C. J.; Van Nevel, C. J. and Demeyer, D. I. 1997. Manipulation of ruminal fermentation. p.523-632. In: The rumen microbial ecosystem. Hobson, P. N., ed. New York, Blackie.

Newbold, J. 1997. Proposed mechanisms for enzymes as modifiers of ruminal fermentation. p.3-7. In: Proceedings of the 16th Florida Ruminant Nutrition Symposium. Gainesville.

NRC - National Research Council. 1996. Nutrient requirements of beef cattle. 7th ed. National Academy Press, Washington, DC.

Nsereko, V. L.; Morgavi, D. P.; Rode, L. M.; Beauchemin, K. A.; McAllister, T. A. 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms in vitro. Animal Feed Science and Technology 88:153-170.

Nussio, L. G.; Campos, F. P. and Lima, M. L. M. 2011. Metabolismo de carboidratos estruturais. p.193-238. In: Nutrição de ruminantes. Berchielli, T. T.; Pires, A. V.; Oliveira, S. G. Funep, Jaboticabal.

Ørskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agriculture Science 92:499-503.

Poppi, D. P. and McLennan, S. R. 1995. Protein and energy utilization by ruminants at pasture. Journal of Animal Science 73:278-290.

Schofield, P.; Pitt, R. E. and Pell, A. N. 1994. Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science 72:2980-2991.

Tavendale, M. H.; Meagher, L. P.; Pacheco, D.; Walker, N.; Attwood, G. T. and Sivajumaran, S. 2005. Methane production from in vitro rumen incubations with Lotus pedunculatus and Megicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. Animal Feed Science and Technology 123-124:403-419.

Theodorou, M. K.; Williams, B. A.; Dhanoa, M. S.; McAllan, A. B. and France, J. 1994. A simple gas production method using a pressure transducter to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology 48:185-197.

Tilley, J. M. A. and Terry, R. A. 1963. A two stages technique for the in vitro digestion of forage crops. Grass and Forage Science 18:104-111.

Vadiveloo, J. 1992. Varietal differences in the chemical composition and in vitro digestibility of rice straw. Journal of Agricultural Science 119:27-33.

Vadiveloo, J. 1995. Factors contributing to varietal differences in the nutritive value of rice straw. Animal Feed Science and Technology 54:45-53.

Van Soest, P. J. 1994. Nutrition ecology of ruminant. 2nd ed. Comstock Cornell University Press, Ithaca, NY.

Van Soest, P. J.; Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597.

Wanapat, M. and Pimpa, O. 1999. Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. Asian-Australasian Journal of Animal Science 12:904-907.

Yang, H. E.; Son, Y. S. and Beauchemin, K. A. 2011. Effects of exogenous enzymes on ruminal fermentation and degradability of alfalfa hay and rice straw. Asian-Australasian Journal of Animal Science 24:56-64.