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The Effect of Different Methods of Processing on Nutritive Value and Degradation of Rice Straw by Rumen Mixed Bacteria

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Abstract: The aim of this study was to evaluate rumen bacteria activity on degradation of untreated Rice Straw (RS) and treated with low temperature steam, sodium Hydroxide (NaOH) and exogenous enzyme using disappearance of Dry Matter (DM) and Neutral Detergent Fiber (NDF) in rumen bacteria culture for 96 h incubation. Treatments were including; untreated RS, RS treated with low temperature steam (120°C for 120 min), RS treated with 80 g kg^{-1} DM NaOH, RS treated with 20 g kg^{-1} DM exogenous enzyme and RS treated with enzyme+NaOH. The result showed disappearance of dry matter after 96 h incubation by rumen bacteria was 60.3, 75.2, 85.3, 81.3 and 96.2 g/100 g for untreated rice straw and treated with steam, NaOH, enzyme and enzyme+NaOH, respectively. Sodium hydroxide, enzyme and steam caused to increase disappearance NDF of rice straw in media culture in compared with the other samples, 96 h after culturing and the highest increase of NDF disappearance was for rice straw treated with enzyme+NaOH (345.3 mg g^{-1}) ($p<0.05$). Therefore, it may be resulted that low temperature steam, exogenous enzyme and NaOH influence the growth and activity of rumen bacteria on rice straw in compared to untreated RS.

Key words: Low temperature steam, sodium hydroxide, exogenous enzyme, rumen bacteria, rice straw, Iran

INTRODUCTION

Rice straw is an abundant by-product of rice production. Recently, there has been increasing interest in exploiting low quality straws for ruminant feeding in many Asian countries. However, the nutritive value of rice straw for ruminants is relatively low due to its high lignocellulosic content, poor palatability and low organic matter digestibility. In addition to it contains a high concentration of silica that acts as a physical barrier preventing bacterial attachment. The information on the effect of low temperature steam, sodium hydroxide (NaOH) and enzyme on NDF degradation of rice straw by rumen bacteria are rare. Many methods have proved successful in disrupting cell wall material e.g., using alkali (Canale *et al*., 1992) and or steam (Castro and Machado, 1990). Under steam conditions, acetyl groups are released from the hemicellulose matrix and suitable levels of cell wall disruption are achieved (Muzzy *et al*., 1983). Steam associated with chemical treatments is known to disrupt lignocellulosics in a way which allows improved utilization of cell wall polysaccharides by rumen microbes (Castro and Machado, 1990). The researchers reported sodium hydroxide may breakdown hemicellulose, hydrolysis the ester bonds between lignin and hemicellulose, expose the cellulose to microbial attachment and carbohydrates more accessible to the action of rumen micro-organisms and improve organic matter digestibility (Goto *et al*., 1993). Also, exogenous enzymes increase digestibility (Euna *et al*., 2006). The aim of this study was to investigate the effect of low temperature steam, enzyme and NaOH on rumen bacteria growth and the *in vitro* disappearance of Dry Matter (DM) and Neutral Detergent Fiber (NDF) of rice straw.

MATERIALS AND METHODS

Samples preparation and culture of rumen anaerobic bacteria:. About 80 g kg^{-1} NaOH on a DM basis was mixed with the rice straw (1.0 mm screen, about 92% DM) for 48 h. Some samples were autoclaved (120°C for 120 min) and then oven-dried at 55°C. Also 20 g kg^{-1} DM exogenous enzyme used for processing rice straw and autoclaved rice straw. The enzyme mixture composition was Cellulase, Xylanase, Beta glucanase, Alpha amylase, Pectinase, Phytase, Protease and Lipase as 0.03, 6.6, 10, 0.7, 0.7, 0.07, 0.5 and 3 MU kg^{-1}, respectively; Bioproton Pty. Ltd. Co. Therefore experimental samples were: untreated Rice Straw (RS), rice straw treated with low temperature Steam (RS1), rice straw treated with 80 g kg^{-1}

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DM NaOH (RS2), rice straw treated with exogenous enzyme (RS3), rice straw treated with enzyme+80 g kg^{-1} DM NaOH (RS4).

Four fistulated sheep which fed 250 g concentrate, 550 g lucerne hay and 200 g wheat straw was used to collect rumen fluid then centrifuged (1000×rpm, 10 min). Supernatant was used to grow bacteria in medium containing fungicides (benomyle, 500 ppm mL^{-1} medium and metalaxyle, 10 mg mL^{-1} medium) under anaerobic conditions at 39°C for 24 h. These isolates were then used as a source of inoculum for culturing bacteria in a serum bottle containing 45 mL of culture medium of rumen bacteria (Caldwell and Bryant, 1966) and 1 g of experimental samples under anaerobic conditions (using three times subculture) at 39°C for 12, 24, 48, 72 and 96 h.

Measurements and statistical analysis: Samples of rice straw used as the substrate of culture media were collected from each bottle after washing twice with distilled water followed by filtration using grade 1 sintered glass crucibles. They were then freeze dried to constant weight for DM determination. The DM disappearance of each sample was calculated as the difference between initial and the residual weight of the dried substrate. Content of NDF of samples were determined from the freeze-dried samples using the method of Van Soest *et al.* (1991) and losses of each sample were calculated as the difference between initial and the residual weight of the dried substrate.

Data of DM and NDF disappearance of medium were analyzed as repeated measurement using the General Linear Model (GLM) procedure of SAS (1996). Duncan's multiple range test was used to compare the means at $p<0.05$.

RESULTS AND DISCUSSION

The data of disappearance of DM and NDF of rice straw treated with low temperature steam, enzyme and NaOH by rumen bacteria culture are shown in Table 1 and 2, respectively. The results showed higher disappearance of DM was observed in the cultures of rice straw treated with enzyme+NaOH that fallowed by rice straw treated with 80 g kg^{-1} DM NaOH (RS2), rice straw treated with exogenous enzyme (RS3) and low temperature Steam (RS1) ($p<0.05$). This result was in agreement with Vadiveloo (2000) by using *in vitro* digestibility of hay leaf and stem. Also the others reported DM and NDF digestion of the straw treated with an alkali was greater than untreated straw (Bas *et al.*, 1989). The current experiment showed that highest disappearance of NDF was observed in the cultures of rice straw treated with

Table 1: The effect of low temperature steam, enzyme and sodium hydroxide on dry matter disappearance of rice straw by rumen bacteria

	DM disappearance (g/100 g DM)						
IT (h)	RS	RS1	RS2	RS3	RS4	SEM	p-value
12	38.2^{e}	45.0^{d}	57.0^{b}	50.0^{c}	67.3^{a}	0.50	<0.0001
24	40.4^{e}	52.1^{d}	60.6^{b}	55.1^{c}	76.7^{a}	0.61	<0.0001
48	52.2^{e}	62.2^{d}	76.5^{b}	65.2^{c}	87.2^{a}	0.62	<0.0001
72	57.1^{e}	73.1^{d}	84.1^{b}	78.1^{c}	92.3^{a}	0.71	<0.0001
96	60.3^{e}	75.2^{d}	85.3^{b}	81.3^{c}	96.2^{a}	0.60	<0.0001

IT, Incubation Time; untreated Rice Straw (RS), rice straw treated with low temperature steam (RS1), rice straw treated with 80 g kg^{-1} DM NaOH (RS2), rice straw treated with 20 g kg^{-1} DM enzyme (RS3), rice straw treated with enzyme+80 g kg^{-1} DM NaOH (RS4); SEM Standard error of mean; Means with different letters within each row differed significantly ($p<0.05$)

Table 2: The effect of low temperature steam, enzyme and sodium hydroxide on neutral detergent fiber disappearance of rice straw by rumen bacteria

	NDF disappearance (mg g^{-1} DM)						
IT (h)	RS	RS1	RS2	RS3	RS4	SEM	p-value
12	98.20^{e}	146.0^{d}	157.0^{b}	154.0^{c}	165.0^{a}	0.73	<0.0001
24	166.4^{e}	217.1^{d}	229.1^{b}	223.1^{c}	236.1^{a}	0.85	<0.0001
48	238.2^{e}	302.2^{d}	316.2^{b}	308.2^{c}	318.2^{a}	0.53	<0.0001
72	249.1^{e}	313.1^{d}	329.1^{b}	3251^{c}	342.1^{a}	0.64	<0.0001
96	252.3^{e}	319.3^{d}	335.3^{b}	329.3^{c}	345.3^{a}	0.42	<0.0001

IT, Incubation time; untreated Rice Straw (RS), rice straw treated with low temperatureSsteam (RS1), rice straw treated with 80 g kg^{-1} DM NaOH (RS2), rice straw treated with 20 g kg^{-1} DM enzyme (RS3), rice straw treated with enzyme+80 g kg^{-1} DM NaOH (RS4); SEM, Standard error of mean; Means with different letters within each row differed significantly ($p<0.05$)

enzyme+NaOH. The alkali solubilizes the inhibitory phenolic compounds and facilitates microbial (*F. Succinogenes*) colonization, increases bacteria populations in cell wall (Chen *et al.*, 2007) and improves of the ruminal degradation plant cell walls (Euna *et al.*, 2006). Gould proposed that alkali react with lignocellulosics to yield partially de-lignified products that are highly susceptible to enzymatic and microbial attach. The enhanced degradability has been ascribed to a solubilisation of total phenolics (Chesson, 1981), arabinoxylans and cellulose (Lindberg *et al.*, 1984) and arising from the cleavage of alkali-labile lignin-carbohydrate linkages (Alexander *et al.*, 1987). It was indicated that 32.5% of the hemicellulose present in the untreated wheat straw was solubilized upon NaOH treatment (Lesoing *et al.*, 1981). In most studies the partial solubilisation of hemicellulose has been demonstrated following alkali treatment (Wadhwa and Makkar, 1995).

The researchers demonstrated the increase in enzymic hydrolysis after steam treatment can be explained by the removal of the hemicellulose but also by the melting and agglomeration of the depolymerized lignin (Toussaint *et al.*, 1991) that caused to increase accessibility and utilization for microbial enzymes (Kling *et al.*, 1987) also cellulose will be more accessible

for rumen microbial enzymes (Castro and Machado, 1990). Any improvement in *in vitro* digestibility of bagasse resulting from steam was due to the formation of water soluble substances. Chaji and Naserian (2006) concluded steam treatment of sugarcane pith significantly increased *in situ* dry matter soluble fraction that solubility might be an estimation of nutrient availability and so digestibility of rumen microbes increased (Dehority and Johnson, 1964). The researchers reported phenolic compounds (furfurals) can be formed during steam treatments and these may be have an inhibitory activity and toxic to the rumen microbes (Kyuma *et al*., 1991). Therefore using lower temperatures with a chemical matter (acid) can achieve comparable cell wall disruption to steam treatment at high temperatures (Castro *et al*., 1994) and results lower amounts of toxic compounds (Clausen and Gaddy, 1983).

The results of present experiment showed that rice straw treated with exogenous enzymes increased disappearance of DM and NDF by rumen bacteria in compared with untreated rice straw. The results of Pinos-Rodriguez *et al.* (2002) showed that enzyme increased *in situ* digestibility of DM and cell wall fractions. Also increased degradation of NDF using exogenous enzymes is reported by Colombatto *et al.* (2003). The researchers reported use of exogenous fibre-degrading enzymes (cellulases) may be improved the degradation characteristics and nutritional value of rice straw. Morgavi *et al.* (2000) indicated that exogenous enzymes work in synergy with rumen microbial enzymes which increases their hydrolytic potential within the rumen and digestibility of plant cell wall. Colombatto *et al.* (2003) concluded that some xylanases and proteases improved *in vitro* degradation of alfalfa hay. Enzymes applied to feed increased solubility of DM and NDF and possibly released more nutrients that were available to support the production of glycocalyx which is produced by bacteria and permits adhesion between bacteria or between bacteria and substrate (Yang *et al*., 1999). Yang *et al*. (1999) reported increased microbial colonization for alfalfa cubes treated with enzyme was likely related to enzyme action. Regarding the factors related to the diet, the effectiveness of fibrolytic enzymes has been shown to vary with forage (Colombatto *et al*., 2003).

CONCLUSION

The result of this experiment suggested that enzyme (20 g kg^{-1} DM) associated with NaOH (80 g kg^{-1} DM) increased *in vitro* degradation and disappearance of DM and NDF of rice straw by rumen bacteria and therefore improved nutritional values of rice straw more than another treatments.

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