

## Evaluation of Digestibility and Nutritional Value of Processed Rice Straw by Bacterial, Enzymatic and Chemical Methods with Gas Production and Chemical Methods

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**Abstract:** This study was carried out to evaluate the effect of rice straw processing by Lactobacillus, Multi enzymes and calcium hydroxide on the digestibility and nutritional value by gas production and chemical methods. Processed for 60 days in anaerobic conditions in the 3 kg tanks were repeated by three times in spray method. The experiment was done in completely randomized design and data were analyzed with SAS and Neway software. After processing the samples dried and Chemical compounds Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Ether Extract (EE), Ash, Crude Fiber (CF), NFC (Non Fiber Carbohydrate), Nitrogen Free Extract (NFE) and Organic Material (OM) and degradation were determined at the times of 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours in gas production method and parameters such as (OMD-SCFA-ME-NEL-CP-DMD) were determined. Each of three processing operation, reduce the amount of cellulose and hemicelluloses in the straw and bacterial processing (%51/2), allocated the least amounts of ADF while enzyme production (%53/8), chemical (%57/9) and control straw (%58/2) maximum allocated the most amounts of ADF. The same trend also has been in the NDF and bacterial processing has the lowest and enzymatic, chemical and control straw have the greatest amount of NDF, respectively. The amount of gas production rate in the bacterial methods was more than the other method and with increasing incubation time, shows upward trend.

**Key words:** Bacterial, chemical, digestibility, enzymatic, gas production, nutritional value, rice straw

### INTRODUCTION

Rice as a staple food of most people and rice straw, as a byproduct, for every one kilogram of rice, is produced about 0/83 kg (Nguyen *et al.*, 1998). In developing countries, particularly in granary Asian countries, straw is the main meal of ruminants (Orskov and McDonald, 1979). Due to suitable climatic conditions, rice cultivation is the most important agricultural activity in northern Iran and about 1100000 tons of rice straw in Guilan (Iran) is produced (Jafari *et al.*, 2003). On the other hand, for economic and environmental reasons and prevent competition between human and animal food, straw consumption should be increased in the final daily food of animals such as sheep (Nguyen, 2000). Rice straw while is cheap can be consider as the huge potential source of energy and dry matter, however, the consumption of lignocelluloses materials are low digestibility in straw. Due to the presence of non-polysaccharide compounds such as folic acid, and some silica by massive food and use it only to food in the bulking digestive tract are used (Nguyen *et al.*, 1998). Being high of structural carbohydrate content

(lignin-cellulose and hemicelluloses) and straw low protein, cause to slow and sometimes lack of food shortages in the rumen and lack of necessary materials in rumen microorganisms (Van soest *et al.*, 1991). Cellulose and hemicelluloses, in addition to a fundamental role in maintaining stability and structure of plants are important food for herbivores (Orskov and McDonald, 1979). Using methods of processing and enrichment was effective in improving the quality and digestibility of fiber and recent developments in fermentation technology and biotechnology provides a possible to produce large amounts of fiber-digesting enzymes and enzymes of Cellulose and Hemicelluloses with microbial origin able to convert cellulose and hemicelluloses to water-dissolved sugars and usable for humans and livestock. Reducing the cost of industrial fermentation process and produce enzymes with optimal performance lead researcher to consider the role and mechanism of production in forages fed to ruminants (Haddad *et al.*, 1998). Enrichmen of rice with urea stated that Nitrogen feed, forage digestibility and intake increased. Increased by 6% lime and 3% Urea increased the consumed dried matter and straw digestibility (Zaman and Owen, 1995). Processing with

Multi-enzyme (cellulose, protease and beta glucanase) in Soya and soymeal increased nutritional digestibility (Nasi, 1991). Enzyme processing is effective to eliminate anti-nutritional factors and increase nutritional digestibility and helping to digestive tract of animals (Bedford, 1993). Impregnated hay packets with enzymes increased bacteria colonies and dry matter digestibility of forage (Yang *et al.*, 1999) and affected gas production in the *in vitro* conditions (Varel *et al.* 1993). The lactobacillus bacteria, as the predominant bacteria in the rumen of young ruminants are one of the most important bacterial fermentation of carbohydrates materials which have an important role in fermentation of glucose into lactate. Lactobacilli are able to convert glucose to 1 and 2 fructose diphosphate, but these bacteria lack of the enzymes 1 and 2 fructose diphosphate aldolase. Lacking 1 and 2 fructose diphosphate aldolase in this bacteria be compensated with the enzyme phosphoketolas. Lactobacillus converts Glucose 6-phosphate to Xilos 5-phosphate trough Pentose phosphate that this material is used as a substrate for the enzymes are phosphoketolas. In this study, the impact of chemical processing (calcium hydroxide), bacteria (lactobacilli) and enzymes (multi-enzyme) was studied on nutritional value and digestibility of rice straw using chemical and gas production.

### MATERIALS AND METHODS

This study was carried out using three adult castrated and equiponderant male sheep (65±2/6 Mean) of Taleshi race and festulated in the Research Institute of Animal Science of Iran (July, 2011). In order to growth and suitable density of microbial population and adoptability, two weeks (daily 8 am and 15 pm) to 60% of tree branches and 40% concentrate were fed in appetite and rock salt and water were freely available (Orskov and McDonald, 1979). Experiment had been done in a complete randomized block design and data obtained

were analysis using ANOVA and Duncan test with SAS and Neway software.

**Enrichment:** After converting rice straw into pieces 3 to 5 cm, treatment sprayed with chemical methods of (40 g lime (calcium hydroxide) in a liter of distilled water) bacteria (1g Lactobacillus in a liter of distilled water) and enzyme (4 g multi-enzyme in a liter of distilled water) and control (1 L of distilled water) per 1 kg dried rice straw completely uniform and were 60 days under anaerobic conditions in the 3 kg container. In order to determine chemical composition and gas production rate, sample dried in an oven with 75°C temperature for 48 h.

**Chemical analysis:** Chemical compounds including dry matter, crude protein (Kjeldahl), crude ash (furnace), Fibre (Fibrotic), Ether Extract (Soxhlet extractor) amount of cell wall without hemicellulose (ADF) and cell wall (NDF) determined based on common method of Van Soest *et al.* (1991) and AOAC (2005).

**Gas production method:** Gas production method based on communication between gas production and fermentation of straw samples processed was performed by in special syringes with suggested methods of Menke and Steingass (1979). Solutions need is include the solution of the original elements, micronutrients; buffer or artificial saliva Rizarosine and regenerative was used. Feeding was done at maintenance extent and rumen fluid was taken before the morning meal. Amount of 200 mg of each sample was placed in 100 ml syringe and 30 ml rumen infiltrated fluid, containing buffer was added to the syringes. Gas production rates was measured and recorded for each of the treatments and replicates at 96, 72, 48, 12, 8, 6, 4, 2, 0 h.

### RESULTS

The results of chemical compounds of processing methods show that an enzymatic method decreases ADF

Table 1: Comparison of chemical compound of processing methods with method of chemical analysis (based on dry material)

	HEM	%OM	%NFE	%NFC	%NDF	%ADF	%Ash	%CF	%EE	%CP
controlled	85.0b	85.0a	49.3ab	9.0a	78.7a	58.2a	15.0b	33.8a	0.06	5.0a
bacteria	87.0a	87.0a	51.4a	5.9b	76.2c	51.2d	13.0b	30.6b	0.07	5.0a
chemical	82.7b	82.7b	47.2bc	9.0a	69.4d	57.9b	17.3a	31.3a	0.07	4.2b
enzyme	86.7a	86.7a	49.3ab	3.8c	76.4b	53.8c	13.3b	30.9b	2.4	4.1b
SEM	0.00	0.62	0.86	0.62	0.22	0.32	0.62	0.48	0.56	0.24
Sig	**	**	**	**	**	**	**	**	**	**

Different letters in each column show significant differences. \*, \*\* is significant respectively in levels of 1% and 5%.

Table 2: Cumulative gas production in vitro (ml/200mg DM) from four trees twigs after incubation for 96 h and the characteristics of fermentation obtained by fitting data for gas production after 2, 4, 6, 8, 12, 24, 48, 72 and 96 h incubation to the equation  $P = a + b(1 - e^{-ct})$

	Incubation time (h)							exponential equation					
	2	4	6	8	12	24	48	72	96	a	b	a+b	c
controlled	1.0a	2.2a	3.7a	5.3a	9.4a	28.8a	44.7a	49.5a	52.6a	3.8	59.0b	62.8	0.03a
bacteria	1.2a	2.1a	3.2a	4.1b	6.0b	19.8b	35.9b	42.8b	48.2b	2.3	61.8ab	64.1	0.02b
chemical	1.2a	2.1a	3.3a	4.3b	6.2b	19.6b	35.8b	42.6b	48.0b	2.1	61.6ab	63.7	0.02b
enzyme	1.0a	2.2a	3.1a	3.9b	6.4b	21.0b	37.9b	44.6b	50.1ab	2.6	63.3a	65.9	0.02b
SEM	0.11	0.20	0.24	0.24	0.36	0.94	0.87	0.81	0.93	1.43	1.08	1.23	0.002
Sig	ns	ns	ns	**	**	**	**	**	**	ns	**	ns	**

a, b and c are constants in the exponential equation  $P = a + b(1 - e^{-ct})$ . a, initial gas production; b, gas production during incubation; a + b, potential gas production; c, rate of gas production, Different letters in each column show significant differences.

\*, \*\* is significant respectively in levels of 1% and 5%.

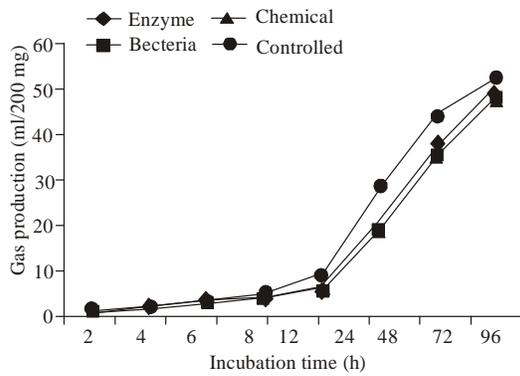


Fig. 1: Cumulative gas production (*in vitro*) over 96 h from the experimental by the straw processing and control

Table 3: The comparison of nutrient digestibility of processing methods (gas test)

	OMD	SCFA	ME	DMD	NEL
controlled	72.9a	6.9a	6.1a	63.0	3.3a
bacteria	63.3b	4.8b	4.9b	65.2	2.5b
chemical	62.6b	4.7b	4.9b	64.9	2.4b
enzyme	60.6b	5.1b	5.1b	66.7	2.6b
SEM	1.34	0.23	0.13	1.08	0.10
Sig	*	**	**	ns	**

Different letters in each column show significant differences. \*, \*\* is significant respectively in levels of 1% and 5%.

and bacterial method increased the rate of crude protein. Also the results show that in all traits measured, methods of processing have a significant effect on straw (Table 1).

The results of gas production show that with increasing fermentation time, amount of gas produced in all experimental treatments was up trend and has increased and have significance difference ( $p \leq 0/05$ ). However in the early hours of incubation, gas production rate of processing methods was not different but then the differences can be observed (Table 2 and Fig. 1).

Organic Matter Digestible (OMD), Short Chain Fatty Acid (SCFA), Metabolism Energy (ME), digestibility of dry matter and Net Energy for Lactating (NEL) showed significant differences ( $p \leq 0/05$ ). Control has the most amounts of SCFA, ME, DMD, NEL and OMD (Table 3).

## DISCUSSION AND CONCLUSION

The results of chemical analysis in Table 1 shows that percent of crude protein (CP) in the control straw and bacterial processing to 5%, has the highest value and is about 4/2% in the enzymatic and chemical treatment. Given the overall low protein of rice straw, processing methods can not have a dramatic impact on it, so that there was not significant difference between different treatments and only in the chemical and enzymatic treatment, has declined due to the use of protein. Van Soest *et al.* (1991) also reported low nitrogen content of the straw and therefore, does not the necessary materials to the rumen microorganisms. Khavidaki *et al.* (2011) in the chemical processing with Sodium hydroxide and a mixture of enzymes as complex (Cellulose and Xylanase) observed that crude protein is reduced which is consistent with survey results. They stated that it is may be due to

lack of lime in the links between straw structural carbohydrates is in short time, so that in a long time, lime which is a chemical material that break and loose the links between cell wall carbohydrates of straw and provide more dissolved materials for microorganisms and raised high levels of protein production. Djajanegara *et al.* (1984) and Garmo (1984) also with compared two methods of soaking and ensiling processing of rice straw with lime stated that due to this issue that lime is a weak alkali with low solubility, so for Silo processing, longer time must be needed. Reduced crude protein of enzyme processing compared to control because of reduced level of enzyme activity in rumen fluid is associated with inactivation and passing through the rumen is associated with the liquid phase (Haristo *et al.*1976). Bacterial processing is not created changes in crude protein which is not conform with the results of Yousiati *et al.* (1995) because bacterial processing had no effect on bond of structural carbohydrates and analysis and production of high protein is not available for microorganisms. Jafari *et al.* (2003) showed that processed rice straw, with rhizous had high protein content and has the ability to convert urea to the protein. Of course, Magne (2001) showed that microorganisms producing microbial protein used structural carbohydrates as an energy source and using nitrogen sources or added nitrogen during processing, convert it to protein. Korad (1996) exposure with rice straw to chemicals such as urea, calcium hydroxide or add protein to the straw increased digestibility and improved feed intake and their degradation. All three methods of production, reduce the amount of cellulose and hemicelluloses in the straw and bacterial processing (%51/2) allocated the least amount and enzyme (%53/8), chemical (%57/9) and control straw (%58/2) processing allocated the most amount of ADF. The same trend also has been in ADF and, respectively, bacterial processing has the lowest and enzymatic, chemical and control straw have the greatest amount of NDF. Stained bacteria during processing of straw will weaken the link between structural carbohydrates such as cellulose, hemicelluloses and some lignin.

Gram-negative bacteria and enzymes in order to remove the problem, that is lacking of nutritional value of straw by dihydro Polymers and labeled lignin in plants lignocelluloses (Haider *et al.*, 1978; Trojanowski *et al.*, 1978). Gram-negative bacteria in the processing of straw reduce its lignocelluloses and improve its digestibility (Richard, 2000) that is completely consistent with the survey results. NDF rate also is reduced in chemical processing (calcium hydroxide). Calcium hydroxide with the weak bonds between the cellulose and hemicelluloses raised the digestibility and accessing to straw carbohydrates and increased the accessibility of bacteria to soluble hydrocarbons. However, Van soest (1991) knew the different responses of materials having high lignocelluloses such as straw to chemical processing from variable rate and different types of lignin from straw. Richard (2000) in processed by three species of bacteria showed that bacterial activity, improve the conditions of lignocelluloses and plant lignin degradation. The enzyme

processing also reduced NDF and ADF. Also, Haider *et al.* (1978) showed that bacterial activity increased degradation and consequently, make digestible Lignin and cellulose material. Survey results are completely consistent with the results of bacterial cellulose and hemicelluloses in rice straw. Lime because of cheap, easy to work with it, safety and lack of need for special equipment are suitable alternative to the profit (Sirohi and Rai, 1995) and importantly, its residues make no toxic in the straw and reduce the need for complementary calcium in the diet (Haddad *et al.*, 1998). However, occurrence of musty and reduce the incidence of diet after enriched with lime are considered as the other problems of this chemical material (Magne, 2001). Zaman and Owen (1995) stated mix of lime and urea is suitable alternative to potash or Ammonia in improving the nutritional value of straw. Also, the results indicated that processing, except for the chemical (lime), everything else increase the organic matter in the straw that showed that bacterial and enzyme activity that is more biologically increase the organic matter but chemical method of processing reduced organic matter.

Gas production with increasing incubation time in the fermentation samples, shows an increasing trend. Gas production in the early hour was less steep and since 12 h later, suddenly in all four treatments, the amount of gas slope too large and will continue until 96 h. This condition can be due to differences in different functions of processing methods. Krishnamorthy *et al.* (1991) indicated that total gas production in the first 24 h of fermentation is produced and the percentage of gas production in the first 2 h after incubation was different in all samples and with increasing incubation time, the difference in gas production will decline. It suggests that differences between foods in the fermentation of biodegradable part, only take place in the early hours of incubation. While in this study, given that the straw has a small protein, so can not be seen many changes at different hours for different processing methods and at high levels not seen significant differences which somewhat confirmed the results of Krishnamorthy *et al.* (1991). Over time, differences in gas production levels are not high. Control and bacterial treatments has high protein compared to the enzymes and chemicals processing and the fermentation and gas production rate can be increased. Singh and Doel (1985) also stated that the high gas production represents high metabolism energy and also fermentable nitrogen and other nutrients needed for microorganisms activities. Dry matter digestibility with increasing of ADF, due to the reduction of soluble Glucosids and increased cell wall is reduced.

With the increase in gas production, digestibility will improved and chemical processing somewhat reduced the production of gas in the high hours of incubation and thus reduces the amount of digestible and this may be due to the low digestibility of calcium hydroxide factor that reduces digestion coefficient and fermentation of processed straw with calcium hydroxide because Calcium

hydroxide did not affect cell wall and materials necessary for microorganisms is not providing. It seems that in the chemical processing with urea and ammonia, due to having high nitrogen and its digestible material compared to calcium hydroxide and having enough nutrients from nitrogen to the rumen microorganisms cause to better break of carbohydrate bonds and in contrast, lime processing of microorganisms due to poor diet and low activity less can cause to break structural carbohydrates bond in rice straw and consequently, fermentation can reduced and reduces the production of gas in 24,48,96 h of incubation. It can be conclude that lime (calcium hydroxide) for microorganisms is not gastronomic and become a limiting factor for the digestion and fermentation by microorganisms. While Taghizadeh *et al.* (2008) have been considered greater amount of gas produced in the first 24 h related to the amount of soluble carbohydrates.

In the enzyme processing, gas production rate of non-soluble part was more and studied enzymes cause to break the bond between structural carbohydrates and higher rates of gas production. And the same, control treatment that was free from any additives for processing, was lowest gas production in non-soluble sector which is due to unavailability of suitable materials for the weak link between structural carbohydrates and consequently, lack of access to the nutrient solution of bacteria and their activity is reduced. In the a+b there was not significant differences between the methods except in enzyme method which again indicating the effect of added enzymes, to make the link between carbohydrates and better adherence of microorganisms to the structural carbohydrates and convert them into butyrate – Acetate-Propionate and high production of gas. In this research, due to lacking of protein, it seems that carbohydrates fermentation into Acetate- Propionate and butyrate caused to produced gas. In the section of gas production rate, there were no significant changes except in control that was a little change which can due to lowing the amount of carbohydrates which easily access for microbial population in rumen fluids which in turn increased the gas production (Deville and Givens, 1998). The rate of Digestible Organic Matter (OMD), Short-Chain Fatty Acids (SCFA), Metabolism Energy (ME) and lactating Net Energy (NE), Dry Matter Digestibility (DMD) in the processing methods were significantly difference. In the Metabolism Energy (ME), the most amounts was related to control and the lowest one was related to chemical and bacterial methods. Metabolism energy (ME) of control treatment was correlated with production gas rate in the h of 24, 48 and 96 of incubations which is consistent with the results of Menke and Steingass (1979).

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