

Influence of Calcium Hydroxide Chemical, Multi-enzyme and Lactobacillus Biologic Processing on Nutritional Value and Digestibility of Rice Straw by Using Nylon-Bags

¹S. Salehpor, ²B. Rasouli and ³A.A. Ghotbi

Agriculture Department, Rasht Branch, Islamic Azad University, Rasht, Iran

Abstract: This research has been conducted to survey the influence of chemical (Calcium Hydroxide), enzyme (Multi-enzyme) and bacterial (lactobacillus) processing on nutritional value and digestibility of rice straw by using nylon-bags and chemical methods. Processing performed for 60 days in anaerobic condition in 3-kg tanks by spraying method in 3 replications. The experiment conducted in complete block design and obtained data were analyzed through SAS and Neway software. After processing period, samples were 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 *in situ* (at times 0, 4, 8, 16, 24, 48, 72 and 96 h) techniques were conducted using 3 fistulated sheep weighted similar to incubation. All three processing methods decreased cellulose and hemicellulose materials in straw and straw in bacterial processing has the least ADF (51.2%) and it has the most amount of ADF in enzyme processing (53.8%), chemical processing (57.9%) and control group (58.2%). The same results obtained about NDF in straw so that straw has the least amount NDF in bacterial processing and it has the most amounts respectively in enzyme, chemical and control group processing. Amount of degradability and disappearance of dry matter in *in situ* technique was more in chemical processing than other processing and it is on an uptrend over passing time of incubation.

Keywords: Nutritional value, nylon-bag (*in situ*), processing, rice straw

INTRODUCTION

Shortage of resources as livestock food, is one of the important barriers to increase livestock production and creating inexpensive and new resources such as processing agricultural byproducts like straw is necessary (Nguyen, 2000). As the major food of peoples of the world, rice is considered a strategic production and rice straw is produced as a byproducts (0.8 kg/1 kg rice) (Nguyen, 1998). Feeding by rice straw does not merely meet all food requirements of ruminants, however in developing countries especially in countries of Asia; straw is the main food of ruminants (Orskov and McDonald, 1979). On the other hand, with regard to economic and biologic issues and in order to prevent from competition between human and livestock food, consumption of straw should be increased in final daily food of livestock such as sheep as much as possible (Nguyen, 2000). As straw has a structure with high carbohydrate (lignin, cellulose and hemicellulose), it cause lower degradation or sometimes no degradation in materials of rumen and low nitrate contents does not give required materials to microorganism of rumen (Van Soest, 1994). Iran has more than 700, 000 hectares which are under cultivation and they annually produce 3, 300, 000 ton straw (Jafari *et al.*, 2009). Regarding that a considerable amount of straw and

stubble are burned by farmers, using methods to increase nutritional value of straw and providing a part of livestock requirements, prevent from environmental effects, destruction and loss of useful microorganism of soil and greenhouse effect in addition of forage production. Today, digestibility of woody materials such as straw of cereals through spraying alkaline solutions such as calcium hydroxide (lime), caustic soda, urea and through microbiological methods such as digesting bacteria and enzymes of cellulose and lignocellulose materials have been under consideration and recent developments in technology and biotechnology of fermentation have provided the possibility of breaking connections between carbohydrates and lignin and therefore carbohydrates become free and digestible and usable for livestock (Partridge and Bedford, 2007). Zaman and Owen (1995) and Pradhan *et al.* (1997) stated that processing straw with lime increases digestibility of organic matter of straw in sheep up to 10 units and energy of straw digestion showed a significant increase. Djajanegara *et al.* (1984) reported that digestibility of dry matter of not-rich and rich rice straw with 4% lime is respectively 38 and 49% in sheep respectively. Edible growth enzymes in combination with multi-enzyme (including Cellulase, Protease and Beta-glucanase) increased degradation of fiber and quality of digestive parameters in sheep feeding

(Jafari, 2007). Decreasing costs of enzyme production process with a delight yield attract researches to consider enzymes role in ruminant nutrition once more (Cone *et al.*, 1997). In addition with regard to putting foods into livestock rumen in *in situ* technique, the possibility of close proximity with natural environment of fermentation is feasible for foods under study so that despite some deficiencies, it could be say that there is not probably any better method for imitating rumen environment (regarding temperature, acidity, buffer and enzymes) (Minson, 1987; Taghizadeh and Farhoomand, 2008). Therefore, the present research deals with the influence of chemical (Calcium Hydroxide), enzyme (Multi-enzyme) and bacterial (Lactobacillus) processing on nutritional value and digestibility of rice straw by using *in situ* technique and chemical method.

MATERIALS AND METHODS

This study was carried out using three adult castrated and equiponderant male sheep ($65 \pm 2/6$ 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 (1 g Lactobacillus in a liter of distilled water) and enzyme (4 g multi-enzyme in a liter of distilled water) and control (1 Liter of distilled water)/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), 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).

Nylon-bags: Measuring dry matter, protein, cell wall (NDF), cell wall without hemicellulose (ADF) disappeared, were conducted through suggestive method by Orskov and McDonald (1979). In Nylon-bag technique (*in situ*), 5-10 g samples were put in bags made of artificial polyester which are indigestible and resistant

against microbial decomposition in rumen with holes of 50 micrometres and dimensions 12×18 cm. Head of bags were blocked by string. Times of incubation were 0, 4, 8, 16, 24, 48, 72 and 96 h and 3 replications were provided per hour for each treatment. After times of incubation finished, the bags were out, washed and completely cleared under cold water flow. Then, bags were put in temperature of 65°C for 24 h. In order to determine its dry matter, they were put in oven with 105°C for 24 hours and percentage of disappeared dry matter and protein computed through following formulas:

Disappeared dry matter (%) = (Bag's weight-Sample or bag's weight after incubation) - (Bag's weight-Sample or bag's weight) $\times 100$

Initial weight of food \times protein (%)

Disappeared protein (%) = (remained protein after incubation (%) \times Remained weight after incubation) - (protein (%) Initial weight of food) $\times 100$

Initial weight of food \times protein (%)

Degradation (%) = (Nutrient after incubation)-(Initial nutrients) $\times 100$

Initial nutrient

It was computed by software Neway and potential degradation through model:

$P = a + b(1 - e^{-ct})$ (Orskov and McDonald, 1979)

- P : (disappeared materials (%) in time t)
- a : The part which is solved quickly (soluble part in time zero)
- b : Sample amount is degraded in time t (Degradable potential part)
- C : Speed of b part's degradation per hour (fixed rate of degradability along time)
- 100-(a+b) : Percent of total non-degradable material in rumen

Data obtained from degradability per hour incubation surveyed through SAS software and based on randomized complete design in 3 replications. Statistical model of the

Design is as: $Y_{ij} = \mu + T_i + e_{ij}$.

Y_{ij} = Amount per observation

μ = Total average

T_i = Effect of treatment

e_{ij} = Experimental error

RESULTS

Analyzing data obtained through chemical method among processing method showed that there are significant differences in all measured traits ($p \leq 0.05$). Also bacterial processing has much better influence on amount of ADF and NDF in compare with other methods.

Table 1: Comparison of chemical compounds of species through chemical degradation (based on dry matter)

	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	**	**	**	**	**	**	**	**	**	**

Columns having different superscripts are significantly different ($p < 0.05$), *, **: is significant respectively in levels of 1 and 5%

Table 2: Average of dry matter degradability of species over incubation time 96 h in "in situ" technique (dry matter (%)), characteristics of degradability for nylon bags after 0, 4, 8, 16, 24, 48, 72 and 96 h incubation and effective degradability of dry matter (ED)

	Incubation time (h)								exponential equation				Effective degradability		
	0	4	8	16	24	48	72	96	a	b	a+b	c	K=2%	K=5%	K=8%
Controlled	13.5b	16.2c	19.6b	31.5b	63.1a	57.4b	60.6b	62.3b	12.1b	57.6b	68.7b	0.02a	44.1b	31.3b	25.9b
Bacteria	11.2c	17.9b	20.1b	25.8c	43.7b	56.5b	59.3c	63.3b	11.3c	56.4b	66.7b	0.03a	44.5b	31.8b	26.2b
Chemical	21.4a	28.9a	36.9a	56.9a	63.1a	73.1a	78.4a	82.4a	21.7a	56.5b	88.2a	0.04a	63.8a	50.9a	44.1a
Enzyme	9.5d	14.6d	20.1b	28.8b	37.5c	49.8c	53.4d	60.4c	10.3d	52.3a	63.6c	0.02a	40.9b	29.3b	24.1c
Sem	0.25	0.17	0.71	1.38	1.1	0.74	0.33	0.66	0.15	0.9	0.68	0.01	0.23	0.35	0.33
Sig	**	*	*	**	**	**	*	**	**	*	**	Ns	**	*	*

a (soluble substances in time 0), b (Fermentable substances) and c (Fixed rate of degradability of part b in time t);

Different letters in each column show significant differences. *, **: is significant respectively in levels of 1% and 5%. Passing rate in speeds of K=2%, K=5%, K=8% is respectively for conditions of treatment, fattening and lactation

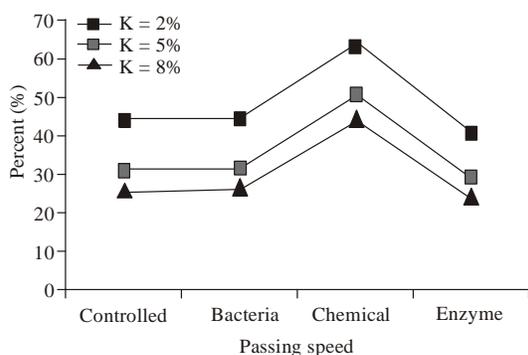


Fig. 1: Degradability of dry matter in in situ technique

Also, the most amount of crude protein belongs to this method (Table 1).

Average of dry matter disappearance in in situ technique was statistically significant ($p \leq 0.05$) at all incubation times in different processing method and it is on an uptrend in each treatment over passing incubation time (Table 2, Fig. 1). Amounts of among processing methods had statistical differences ($p \leq 0.05$). Among treatments, the most amount of belongs to chemical processing and the least amount belongs to enzyme processing.

However, amounts of b and c did not have statistical differences in all three processing methods ($p \geq 0.05$) (Table 2). Regarding effective degradability of dry matter there were statistical differences ($p \leq 0.05$) between processing methods. The most effective digestibility of dry matter in treatment, fattening and lactating conditions belongs to chemical method and its least amount belongs to enzyme method (Table 2 and Fig. 2).

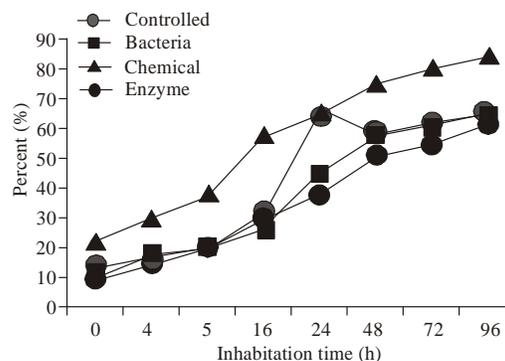


Fig. 2: Effective degradability of dry matter

DISCUSSION AND CONCLUSION

Results of chemical degradation in Table 1 show that percentage of crude protein in straw of control group and in bacterial processing is 5% which is the most amount and it is approximately 4.2% in enzyme and chemical processing. Regarding to general shortage of protein in rice straw, processing methods could not have considerable influence on them so that this difference is not so considerable between different treatment and it is decreased only in chemical and enzyme treatment due to using protein. Ho *et al.* (2008) reported that nitrate content of straw is low too and it is a reason that required materials are not given to microorganisms of rumen. During chemical processing through sodium hydroxide and enzyme complex (Cellulase and Xylanase), Karimi (2007), observed that amount of crude protein is decreased which is consistent with the result of the present research while it is not consistent with the results

which say “crude protein of processed wheat straw with lime is decreased”. Regarding that lime is a weak alkali with low solubility; therefore, longer time is needed for effective processing of lime through chemical method so that, Schiere and Ibrahim (1989) and Chenost and Kayouli (1997) stated that ineffectiveness of lime in connection between structural carbohydrates of straw is related to short time of processing and they said that during long times, lime breaks and weakens connections between carbohydrates of cell wall of straw so that more soluble materials are given to microorganism of rumen and therefore protein is produce in high level. Regarding to have enough time for processing (two months) and effecting on structural carbohydrate reduction, such condition could be observed. Other results also reported increase of digestibility and improvement of palatability and degradability through processing rice straw by calcium hydroxide. Of course, Ho *et al.* (2008), believes that different responses to chemical processing by materials with high lignocellulose such as straw are due to variable amount and different kind of lignin and similar materials of straw. Enzyme processing decreases crude protein in compare with control treatment but bacterial processing does not change it. These results are consistent with the results obtained by Wang *et al.* (2001) who said bacterial processing and time of bacterial processing were not effective on connections of structural carbohydrates and possibility for conducting degradation and producing high protein were not provided for microorganism. In processing 3 species of bacteria, Richard (2000) showed that bacterial activity improves degradation of lignocellulose and plant lignin. Of course studies conducted by Moslemioon (2002), show that microorganisms which produce microbial protein, use structural carbohydrates as energy resources and change them to protein resources through existing or added nitrogen resources during processing. All three methods of processing decrease amount of cellulose and hemicellulose materials and bacterial processing (51.2%), enzyme processing (53.8%), chemical processing (57.9%) and control treatment (58.2%) have respectively the least to the most amount of ADF and NDF. As lime is inexpensive and safe and as it could be simply used and it does not need any special equipment, it is a suitable replacement for soda (Sirohi and Rai, 1995) and more important is that rest of it does not poison straw and it decreases need of calcium complementary in ration (Haddad *et al.*, 1998). Of course mildew and decrease in food consumption after riching by lime are other problems of this chemical substance (Magne, 2001). Zaman and Owen (1995) stated that compound of lime and urea is a proper replacement for soda or ammonia in order to improve nutritional value of straw. Organic matter in all processing method except in calcium hydroxide processing increases organic matter in straw due to enzyme and bacterial activities which are more biologic. Average of dry matter disappearance in all three methods

has significant difference and it is on an uptrend over passing time. This phenomenon is justifiable due to changes in population of microorganisms in rumen after feeding and effect of food (Najaf, 2006). Calcium Hydroxide processing at times of incubation has the most amount of dry matter degradability. Even time of incubation becomes longer, degradability of dry matter in chemical treatments increases. The reason for increasing dry matter degradability at all times specially at final hours is breaking up connections of cellulose, hemicellulose and lignin. Also, as lime absorbs humid, dry matter degradability increases over passing time. Results of lime processing are consistent with results obtained by Olivers *et al.* (1993), Nguyen (2000), Pradhan *et al.* (1997) and Sirohi and Rai (1995). Dry matter degradability in bacterial method enjoys a better condition in compare with enzyme processing. Haider *et al.*, (1992) and Robinson and Crawford (1978) stated that this condition is due to effect of bacteria which degrades labeled dehydropolymers and as a result lignin and lignocelluloses become degraded so that it effects on digestibility and palatability and so digestibility of rice straw increases. These results are consistent with the results of the present research. Also, processing causes that existing bacteria in rumen attack more and easily toward structural carbohydrates so that increase digestibility and palatability of rice straw (Bod'a, 1990). The most amount of dry matter degradability is related to chemical method and the least amount is related to enzyme processing. Chemical processing (Calcium hydroxide) has the most amounts of soluble substances (Nguyen, 2000). In all processing methods when passing rate increases (2, 5, 8%), disappearance is on a downtrend and amount of degradation decreases. Results show that the most effective degradability in three conditions of treatment, fattening and lactating (K = 2 %, K = 5% and K = 8%) is related to chemical processing and the least amount is related to enzyme processing. This condition could be always observed in amounts of materials in *in situ* technique (Dry matter degradability) which shows interactions in fermentation and degradability of dry matter. As it could be observed, degradability of dry matter is depended on time period that a sample remains in rumen and even passing rate is more, effective degradability decreases due to less compatibility and less effectiveness of microorganism of rumen (Gosselink *et al.*, 2004).

REFERENCES

- AOAC, 2005. Official Methods of Analysis, AOAC International. 18th Edn., Gaithersburg, USA.
- Bod'a, K., 1990. Nonconventional Feedstuffs in the Nutrition of Farm Animals. Developments in Animal and Veterinary 23. Elsevier, Amsterdam-Oxford-NewYork, Tokyo.

- Chenost, M. and C. Kayouli, 1997 Roughage Utilization in Warm Climates. FAO Animal Production and Health Paper 135, Rome.
- Cone, J.W., H.A. Van Gelder and F. Driehuis, 1997. Description of gas production profiles with a three phasic model. Anim. Feed Sci. Tech., 66: 31-45.
- Djajanegara, A., P.T. Doyle and B. Molina, 1984. Calcium hydroxide treatment of wheat straw. Cited in (Gosselink. *et al.*, 2004).
- Gosselink, J.M.J., J.P. Dulphy, C. Poncet, S. Tamminga and J.W.A. Cone, 2004. Comparison of *in situ* and *in vitro* methods to estimate *in vivo* fermentable organic matter of forages in ruminants. NJAS, 52-1: 29-45.
- Haddad, S.G., R.G. Grant and S.G. Kachman, 1998. Effect of wheat straw treated with alkaline on ruminal function and lactation. J. Dairy Sci., 81: 1956-1965.
- Haider, Y., Z. Kerem and B. Gorodeki, 1992. Biodegradation of lingocellulose agriculture waste by *pleurotus ostreatus*. J. Biotech., 30: 133-139.
- Ho, T.T., Ngo Van Man and T.R. Preston, 2008. Performance of young cattle fed rice straw sprayed with mixture of urea and molasses supplemented with different levels of cassava leaf meal. Livestock Res. Rural Dev., 20 (suppl.).
- Jafari, M.A., 2007. Study on Kinetic degradability of cell wall of processed rice husk by biologic method (cultivating 4 species of mushrooms *Pleurotus*) through insaco technique. The First National Conference of Modern Technologies in Agriculture and Natural Resources.
- Jafari, K.K., M.R. Jafari and M.A. Jafari, 2009. Surveying influence of chemical and biologic processing on digestibility of soybean straw through *in vitro* technique. The First National Conference of Modern Technologies in Agriculture and Natural Resources.
- Karimi, A., 2007, Educational and Promoting Journal of Processing Straw and Stubble. Publication of Managing Public Promotion and Participation of Fars Agriculture. pp: 10-8.
- Magne, M., 2001. Recent Trends in Upgradating and Utilization of Crop Residues for Animal Feeding. Department of Animal Science, Agriculture University, Norway.
- Minson, D.J., 1987. Estimation of the Nutritive Value of Tropical Pasture, In: Wheeler, J.L., C.J. Pearson and G.E. Roberts, (Eds.), Tropical Pasture, their Production, use and Management, Australian Wool Corporation, pp: 415-422.
- Moslemioon, M.T., 2002. Surveying effects of using different levels of straw with adding enzyme or without enzyme on production ability of broiler chicken. MS Thesis, Organization of Agricultural Research and Education, Center of Agricultural Higher Education.
- Najaf, N.B., 2006. Surveying on nutritional value of trifolium and some concentrated processed materials through techniques *in vivo*, *in vitro*, *in situ* and gas production. MS Thesis, Boo Ali Sina University, Faculty of Agriculture.
- Nguyen, X.T., 1998. The need for improved utilisation of rice straw as feed for ruminants in Vietnam: An overview. Livestock Res. Rural Dev., 10(2).
- Nguyen, V.T., 2000. Treatment and supplementation of rice straw for ruminant feeding in vietnam. Ph.D. Thesis, Hanoi Agriculture University Scientiarum.
- Olivers, B.A., R.A. Brittom and T.J. Klopfenstein, 1993. Ammonia and calcium hydroxide treatment of maize strover. Anim. Feed Sci. Thech., 44: 59-72.
- Orskov, E.R. and I. McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurement weighed according to rate of passage. J. Agric. Sci., 92: 499-503.
- Partridge, G. and M. Bedford, 2007. Engymes in Farm Animal Nutriion. CABI Publishing, ISBN: 0851993931.
- Pradhan, R., H. Tobioka and I. Tasaki, 1997. Effect of moisture content and different levels of additives on chemical composition and *in vitro* dry matter digestibility of rice straw. Anim. Sci. Tech. (Japan), 68(3) : 273-284.
- Richard, T., 2000. The Effects of Lignin on Biodegradability. Cornell Composting. Science and Engineering., Ithaca, NY. pp: 1-139
- Robinson, I.E. and R.L. Crawford, 1978. Degradation of ¹⁴C, Labeled lignins by *Bacillus megaterium*. FEMS Microbiol. Lett., 4: 301-302.
- Schiere, J.B. and M.N.M. Ibrahim, 1989. Feeding of Urea-Ammonia Treated Rice Straw. Pudoc Wageningen, Netherlands.
- Sirohi, S.K. and S.N. Rai, 1995. Associative effect of lime plus urea treatment of paddy straw on chemical composition and *in vitro* digestibility. Indian J. Anim. Sci., 65(12): 1346-1351.
- Taghizadeh, A. and P. Farhoomand, 2008. Scientific Feeding of Dairy-Cow. Jahad-e- Daneshgahi Institute of Western Azarbaijan, Uromieh Branch.
- Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. 2nd Edn., Cornell University Press, USA.
- Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
- Wang, Q., K. Yamabe, J. Narita, M. Morishita, Y. Ohsumi, K. Kusano, Y. Shirai and H.I. Ogawa, 2001. Suppression of growth of putrefactive and food poisoning bacteria by lactic acid fermentation of kitchen waste. Process Biochem., 37: 351-357
- Zaman, M.S. and F. Owen, 1995. The effect of calcium hydroxide and urea treatment of barley straw on chemical composition and digestibility *in vitro*. Anim. Feed Sci. Tech., 51(1): 165-171.