

IN VITRO CELLULOSE DISAPPEARANCE OF BREWERY WASTE INCORPORATED PADDY STRAW BASED FEEDS IN RUSITEC

S. Senthilkumar*

Department of Animal Nutrition
College of Veterinary and Animal Sciences
Kerala Veterinary and Animal Sciences University
Mannuthy, Thrissur, Kerala, India

ABSTRACT

An *in vitro* experiment was conducted to study the *in vitro* cellulose disappearance of the following seven experimental feeds such as 1) Control feed; 2) Experimental feed (75%) + Fresh brewery waste (25%) (EFFBW); 3) Experimental feed (75%) + Dried brewery waste (25%) (EFDBW); 4) Fresh brewery waste (FBW); 5) Dried brewery waste (DBW); 6) Paddy straw (PS) and 7) Brewery waste incorporated paddy straw (BWIPS) incubated for 2, 6, 12, 24, 48 and 72 hours in Rumen Simulation Technique (RUSITEC). During initial incubation periods (2, 6, 12 and 24 hours), control feed, EFFBW, EFDBW and FBW showed higher *in vitro* cellulose disappearance than other experimental feeds, whereas, during later incubation periods (48 and 72 hours), the DBW, paddy straw and BWIPS showed higher *in vitro* cellulose disappearance than other experimental feeds incubated in RUSITEC. Among the feeds experimented in RUSITEC, disappearance of cellulose was more in the control feed, EFFBW and EFDBW when compared to other experimental feeds. Similarly, the cellulose disappearance was more in BWIPS compared to paddy straw. The *in vitro* cellulose disappearance rates at all incubation periods were higher for FBW than those for DBW.

Keywords: Brewery waste, paddy straw, cellulose disappearance, *in vitro*, RUSITEC

INTRODUCTION

Brewery waste is a byproduct of ethanol industry which uses cereal grains as feed stock. Distiller's grain has a moderate content of protein and high level of crude fibre which makes it an attractive ingredient to be used as ruminant feed (Rasco *et al.*, 1989).

Among the various cereal crop residues, paddy straw (*Oryza sativa*) is the main roughage source for majority of cattle and buffaloes reared in Indian small holdings. However, the nutrient digestibility of straw is very poor. Knowledge on disappearance / degradability of brewery waste incorporated with paddy straw would throw more light on its usefulness and would help to formulate complete feed for dairy cattle. The present study was undertaken

*Corresponding author email: annsenthil@gmail.com

to evaluate the *in vitro* cellulose disappearance of brewery waste incorporated paddy straw-based feeds using Rumen Simulation Technique (RUSITEC).

Table 1.
Per cent ingredient composition of the control and experimental concentrate mixtures used

Ingredient	Concentrate mixture (%)	
	Control	Experimental
Yellow Maize	37.00	40.00
Groundnut Cake	29.00	21.50
Wheat bran	30.50	10.00
Brewery waste	0.00	25.00
Mineral Mixture*	1.00	1.00
Salt	1.00	1.00
Shell Grit	1.50	1.50

To every 100 kg of concentrate mixture 20 grams of Nicomix AB₂D₃K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A-82500 I.U, Vitamin D₃-12000 I.U, Vitamin B₅-50 mg, Vitamin K-10 mg per gram was added.

*Mineral mixture supplied by Kerala Feeds Ltd. Kerala, containing Calcium (minimum) 20 per cent, Phosphorus (minimum) 12 per cent, Magnesium (minimum) 5 per cent, Iron (minimum) 0.4 per cent, Copper (minimum) 0.1 per cent, Zinc (minimum) 0.8 per cent, Manganese (minimum) 0.12 per cent, Cobalt (minimum) 0.012 per cent, Iodine (minimum) 0.026 per cent, Sulphur 1.8-3 per cent, Arsenic (maximum) 7 ppm, Lead (maximum) 20 ppm and Flourine (maximum) 0.07 per cent

MATERIALS AND METHODS

Per cent ingredient composition of the control and experimental concentrate mixtures used are presented in Table 1. The dry matter (DM) composition of Control feed, Experimental feed, Brewery waste, Paddy straw and Brewery waste incorporated paddy straw were carried out as per standard procedure (AOAC, 1990). The cellulose content of control feed, experimental feed, brewery waste, paddy straw and brewery waste incorporated paddy straw were carried out as per AOAC (1990).

The *in vitro* cellulose disappearance of the seven experimental feeds viz., control feed, experimental feed with fresh brewery waste (EFFBW), experimental feed with dried brewery waste (EFDBW), fresh brewery waste (FBW), dried brewery waste (DBW), paddy straw (PS) and brewery waste incorporated paddy straw for 3 days (BWIPS) was determined using the rumen simulation technique (RUSITEC) described by Czerkawski and Breckenridge (1977). The *in vitro* cellulose disappearance of experimental feeds was studied at 0, 2, 6, 12, 24, 48 and 72 hours of incubation in RUSITEC and the experiment was replicated.

Each RUSITEC experiment totally consisted of 7 days adaptation period followed by collection period. Loss in weight of nylon bag after 0, 2, 6, 12, 24, 48 and 72 hours of incubation in RUSITEC followed by washing and drying was recorded to calculate *in vitro* cellulose disappearance.

$$\text{In vitro cellulose disappearance} = \frac{(\text{weight of bag with cellulose before incubation}) - (\text{weight of bag with cellulose after incubation})}{\text{weight of sample}} \times 100$$

The data were analyzed statistically as per the standard statistical methods given by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The dry matter (DM) composition of control feed, experimental feed, brewery waste, paddy straw and brewery waste incorporated paddy straw were 95.15 ± 0.56, 94.76 ± 0.26, 29.15 ± 0.43, 90.35 ± 0.81 and 78.11 ± 0.63 per cent, respectively. The

cellulose content of control feed, experimental feed, brewery waste, paddy straw and brewery waste incorporated paddy straw were 4.22 ± 0.24, 6.46 ± 0.28, 13.14 ± 0.35, 34.56 ± 0.52 and 29.21 ± 0.56 per cent, respectively on dry matter basis.

Paddy Straw

The chemical composition of brewery waste, paddy straw and brewery waste incorporated paddy straw were presented in Table 2.

Table 2. Per cent chemical composition of the brewery waste, paddy straw and brewery waste incorporated paddy straw* (on DM basis)

Nutrients	Brewery waste	Paddy straw	Brewery waste incorporated paddy straw
Dry matter	29.15 ± 0.43	90.35 ± 0.81	78.11 ± 0.63
Crude protein	24.34 ± 0.60	4.42 ± 0.18	9.41 ± 0.44
Ether extract	5.19 ± 0.18	0.86 ± 0.05	1.94 ± 0.13
Crude fibre	19.62 ± 0.31	34.19 ± 0.61	30.55 ± 0.57
Total ash	5.76 ± 0.14	10.64 ± 0.25	9.42 ± 0.18
Nitrogen free extract	45.07 ± 0.79	49.89 ± 0.64	48.69 ± 0.61
Organic matter	94.25 ± 0.14	89.36 ± 0.25	90.58 ± 0.18
Acid insoluble ash	4.42 ± 0.15	8.76 ± 0.20	7.68 ± 0.18

* Mean of four values ± SE

Paddy straw had higher hemicellulose, cellulose and lignin content than those reported by Rai and Mudgal (1987), Rai *et al.* (1989), Reddy *et al.* (1993), Bae *et al.* (1997), Reddy (1997), Mishra *et al.* (2000), Nguyen and Uden (2001), Singh *et al.* (2001), Sohane and Singh (2001), Singh *et al.* (2002) and Nader and Robinson (2008). However, Adeloye (2001) reported much higher hemicellulose value of 41.64 per cent and Gangwar and Sharma (2001) reported lower hemicellulose value of 16.6 per cent and Rangnekar *et al.* (1982) reported higher lignin value of 11.2 per cent, while Garg *et al.* (2002) reported lower lignin value of 2.70 per cent for paddy straw. Paddy straw contained high acid insoluble ash (8.76 per cent), but the lignin content was lower (5.25 per cent). Rangnekar *et al.* (1982) and Reddy *et al.* (1993) have also reported similar findings in paddy straw. It might be possibly due to the ash especially silica incrustation which may act like lignin components by blocking the cell wall structure of paddy straw and rendering it impermeable (Vansoest and Jones, 1968). Differences in the chemical composition of paddy straw obtained in present study may be attributed to the differences in the variety of paddy straw.

Brewery waste and BWIPS

The cellulose content of brewery waste was 13.14 ± 0.35 per cent and that of brewery waste incorporated paddy straw was 29.21 ± 0.56 per cent. The lignin content of paddy straw was 5.25 ± 0.27 per cent. The lignin content of brewery waste and brewery waste incorporated paddy straw was 7.12 ± 0.26 and 5.72 ± 0.28 per cent, respectively. The results indicated that the brewery waste had moderately high lignin (7.12 ± 0.26 per

cent) content when compared with the value of 4 to 5 per cent as reported by Murdock *et al.* (1981) and Depeters *et al.* (1997). The differences in the chemical composition and fibre fractions of brewery waste observed in the present study may be attributed to the nature of grain used for beer making, differences in the manufacturing process and differences in the analytical techniques employed (Senthilkumar *et al.*, 2010). The low nutritive value of paddy straw was attributed to the presence of indigestible lignin, silica and the manner in which it was bound to digestible cellulose and hemicellulose. This depressed the voluntary feed intake and digestibility of straw (Wanapat, 1984 and Doyle *et al.*, 1986). Doyle (1989) reported the supplementation of nutrients that are limiting in paddy straw as the most practical means of improving the nutrient utilization from paddy straw. Chowdhury (2001) reported that the nutritional constraints of paddy straw were low content of readily fermentable energy, nitrogen, minerals and vitamins. Compared to dried brewery waste (DBW), the fresh brewery waste (FBW) have low dry matter content, are a reasonable protein source, and have a high content of total digestible nutrients (TDN) due to digestibility of available fibre (Hersom, 2006). The moisture content of fresh brewery waste can affect the level of intake in cattle, particularly when it is fed in combination with fresh forages or silage. Schingoethe *et al.* (1988) also reported that for every 10% increase in dietary moisture content, the dry matter intake decreases by 0.2 pound per 100 pound of body weight, due to the response to gut fill and distention caused by the structural volume of plant water held within the cell (Balch and Campling 1962; Van Soest 1982). Also, because NDF (cellulose, hemicelluloses, etc) has a slower rate of fermentation and degradation within the rumen, forages and

feeds (BWIPS) that contain higher levels of NDF can limit intake due to their greater filling effect (Allen, 1996).

***In vitro* cellulose disappearance**

The percentage *in vitro* disappearance of cellulose of control feed, experimental feed with fresh brewery waste (EFFBW), experimental feed with dried brewery waste (EFDBW), fresh brewery waste (FBW), dried brewery waste (DBW), paddy straw (PS) and brewery waste incorporated paddy straw (BWIPS) incubated for 0, 2, 6, 12, 24, 48 and 72 hours in RUSITEC are given in Table 3 and is illustrated in Fig. 1.

The *in vitro* cellulose disappearance of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS was 18.73, 14.42, 13.96, 13.63, 11.93, 8.43 and 12.10 per cent, respectively at 2 hours of incubation. The *in vitro* cellulose disappearance at 24 hours incubation period was 43.70, 34.53, 31.80, 32.85, 30.88, 22.82 and 31.01 per cent for control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS, respectively in RUSITEC.

The *in vitro* cellulose disappearance was 52.50, 51.61, 50.99, 46.24, 42.19, 30.36 and 37.84 per cent, respectively at 72 hours of incubation in the RUSITEC.

It can be seen that among the experimental feeds, control feed, EFFBW and EFDBW showed relatively higher *in vitro* disappearance of cellulose in RUSITEC. Paddy straw showed lowest *in vitro* disappearance of cellulose. However, the BWIPS showed slightly higher *in vitro* disappearance of cellulose than the paddy straw. The FBW and DBW showed intermediary *in vitro* disappearance of cellulose at all incubation periods was higher for FBW than the DBW. Durand *et al.* (1988) observed a significant improvement in the *in vitro* (RUSITEC) hemicellulose and cellulose degradability when wheat straw was treated with sodium hydroxide. Similarly, addition of cationomycin (Ionophore) to fescue hay based pelleted diet showed increased *in vitro* (RUSITEC) NDF, ADF, hemicellulose and cellulose degradability (Bogaert *et al.*, 1989). Bogaert *et al.* (1990) found significant

Table 3. *In vitro* cellulose disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

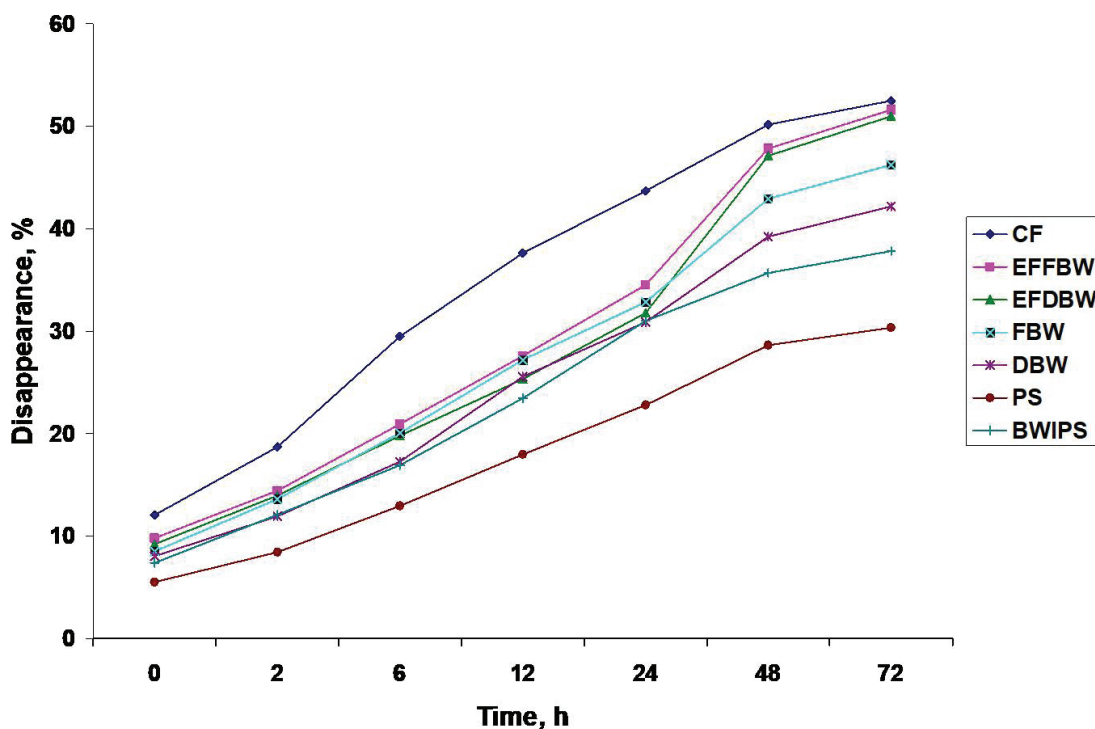
Treatments	Cellulose Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	12.05	18.73	29.50	37.62	43.70	50.16	52.50
Experimental feed with fresh brewery waste (25%)	9.82	14.42	20.95	27.58	34.53	47.81	51.61
Experimental feed with dried brewery waste (25%)	9.22	13.96	19.82	25.34	31.80	47.13	50.99
Brewery waste (fresh)	8.53	13.63	20.09	27.21	32.85	42.93	46.24
Brewery waste (dried)	8.04	11.93	17.28	25.55	30.88	39.24	42.19
Paddy straw	5.52	8.43	12.97	17.97	22.82	28.63	30.36
Brewery waste incorporated paddy straw	7.41	12.10	16.93	23.45	31.01	35.69	37.84

improvement in the *in vitro* (RUSITEC) hemicellulose degradability of alfalfa or wheat straw as a result of monensin addition. Addition of fibrolytic enzymes (30000 nova cellulase and 10000 nova xylanase units per kg DM) significantly improved the *in vitro* (RUSITEC) cellulose and hemicellulose disappearance of orchard grass hay (Dong *et al.*, 1999). Sliwinski *et al.* (2002) found no significant difference in the 48 hour *in vitro* (RUSITEC) NDF, ADF and hemicellulose degradability of basal diet (containing grass silage, barley grain and grass hay) compared to those with soyabean meal based diet. A higher *in vitro* cellulose disappearance

(39.51 per cent) of paddy straw than that is obtained in the present study (30.36 per cent) in RUSITEC was reported by Senthilkumar *et al.* (2007).

Among the feeds experimented in RUSITEC, disappearance of cellulose was more in the control feed, EFFBW and EFDBW when compared to other experimental feeds. Similarly, the cellulose disappearance was more in BWIPS compared to paddy straw. The *in vitro* cellulose disappearance at all incubation periods was higher for FBW than the DBW. A low *in vitro* cellulose disappearance of 30.36 per cent was recorded at 72 hours of incubation for paddy straw,

Fig.1. In vitro cellulose disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %



whereas the BWIPS had improved *in vitro* cellulose disappearance of 37.84 per cent at 72 hours of incubation.

ACKNOWLEDGEMENTS

The author thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, Kerala Agricultural University, Thrissur, Kerala, India for providing the facilities and financial support provided to carry out this research.

REFERENCES

- Adeloye, A. (2001). Improving the nutritive value of rice straw by ensiling with different additives. *Indian Journal of Animal Sciences*, **71**: 58-61.
- AOAC (1990). *Official Methods of Analysis*, Fifteenth edition. Association of Official Analytical Chemists. Washington D.C, pp. 587.
- Allen, M. S. (1996). Physical constraints on voluntary intake of forages by ruminants. *Journal of Animal Science*, **74**:3063-3075.
- Bae, H.D., McAllister, T.A., Kokko, E.G., Leggett, F.L., Yanke, L.J., Jakober, K.D., Ha, J.K., Shin, H.T. and Cheng, K.J. (1997). Effect of silica on the colonization of rice straw by ruminal bacteria. *Animal Feed Science and Technology*, **65**: 165-181.
- Balch, C. C. and Campling, R.C. (1962). Regulation of voluntary food intake in ruminants. *Nutrition Abstracts and Reviews*, **32**:669.
- Bogaert, C., Gomez, L., Jouany, J.P. and Jeminet, G. (1989). Effects of the ionophore antibiotics lasalocid and cationomycin on ruminal fermentation *in vitro* (RUSITEC). *Animal Feed Science and Technology*, **27**: 1-15.
- Bogaert, C., Jouany, J.P. and Jeminet, G. (1990). Effects of the ionophore antibiotics moensin, monensin-propionate, abierixin and calcimycin on rumen fermentations *in vitro* (RUSITEC). *Animal Feed Science and Technology*, **28**: 183-197
- Chowdhury, S.A. (2001). Effect of graded levels of cottonseed cake supplementation on intake, nutrient digestibility, microbial N yield of growing native (*Bos indicus*) bulls fed rice straw. *Asian-Australian Journal of Animal Sciences*, **14**: 326-332.
- Czerkawski, J.W. and Breckenridge, G. (1977). Design and development of a long term rumen simulation technique (RUSITEC). *British Journal of Nutrition*, **38**: 371-384.
- Depeters, E.J., Fadel, J.G. and Arosemena, A. (1997). Digestion kinetics of neutral detergent fibre and chemical composition within some selected by-product feed stuffs. *Animal Feed Science and Technology*, **67**: 126-140.
- Dong, Y., Bae, H.D., McAllister, T.A., Mathison, G.W. and Cheng, K.J. (1999). Effects of exogenous fibrolytic enzymes, α -bromoethanesulfonate and monensin on digestibility of grass hay and methane production in a rumen simulation (RUSITEC) system. *Canadian Journal of Animal Science*, **79**: 491-498.
- Doyle, P.T., Devendra, C. and Pearce, G.R. (1986). Rice straw as a feed for ruminants. *International Dev. Program of Australian Universities and Colleges*

- (IDP), Canberra, VII: 117.
- Doyle, P.T. (1989). Supplementation of rice straw with dry Leucaena. *Australian Journal of Agricultural Research*, **40**: 381-394.
- Durand, M., Dumay, C., Blaumatin, P. and Morel, M.T. (1988). Use of the rumen simulation technique (*RUSITEC*) to compare microbial digestion of various byproducts. *Animal Feed Science and Technology*, **21**: 197-204.
- Gangwar, B.S. and Sharma, S. D. (2001). Nutrient utilization in cross bred heifers fed sulphur supplemented rice straw based diet. *Indian Journal of Animal Nutrition*, **18**: 180-184.
- Garg, M.R., Sherasia, P.L., Bhanderi, B.M., Gulati, S.K. and Scott, T.W. (2002). Effect of feeding rumen protected nutrients on milk production in cross bred cows. *Indian Journal of Animal Nutrition*, **19**: 191-198.
- Hersom, M. J. (2006). By-product feed utilization for forage diets. In: 55th Annual Florida Beef Cattle Short Course, Gainesville: University of Florida Institute of Food and Agricultural Sciences. pp 5-14.
- Mishra, A.S., Chaturvedi, O.H., Khali, A., Prasad, R., Santra, A., Misra, A.K., Parthasarathy, S. and Jakhmola, R.C. (2000). Effect of sodium hydroxide and alkaline hydrogen peroxide treatment on physical and chemical characteristics and IVOMD of mustard straw. *Animal Feed Science and Technology*, **4**: 257-264.
- Murdock, F.R., Hodgson, A.S., Robert, E. and Riley, J.R. (1981). Nutritive value of wet brewers grains for lactating dairy cows. *Journal of Dairy Science*, **64**: 1826-1832.
- Nader, G.A. and Robinson, P.H. (2008). Effects of maceration of rice straw on voluntary intake and performance of growing beef cattle fed rice straw based rations. *Animal Feed Science and Technology*, **146**: 74-86.
- Nguyen, V.T. and Uden, P. (2001). Effect of urea-molasses cake supplementation of swamp buffaloes fed rice straw or grasses on rumen environment, feed degradation and intake. *Asian-Australian Journal of Animal Sciences*, **14**: 631-639.
- Rai, S.N. and Mudgal, V.D. (1987). Associative effect of NaOH and steam pressure treatment on chemical composition of rice straw and its fibre digestibility in rumen. *Indian Journal of Animal Nutrition*, **4**: 5-11.
- Rai, S.N., Walli, T.K. and Gupta, B.N. (1989). The chemical composition and nutritive value of rice straw after treatment with urea or *Coprinus timetarius* in a solid state fermentation system. *Animal Feed Science and Technology*, **26**: 81-92.
- Rangnekar, D.V, Badve, V.C., Kharat, S.T., Sobale, B.N. and Joshi, A.L. (1982). Effect of high pressure steam treatment on chemical composition and digestibility *in vitro* of roughages. *Animal Feed Science and Technology*, **7**: 61-70.
- Rasco, B.A., Borhan, M. and Owusu-Ansah, Y. (1989). Effects of drying techniques and incorporation of soluble solids on the chemical composition and color of distiller's grain products. *Cereal Foods World*, **34**: 346-349.

- Reddy, D.V., Krishna, N., Naidu, K.N. and Reddy, R.R. (1993). Effect of substituting conventional concentrate mixture by *Leucaena leucocephala* leaf fodder in crossbred calves fed rice straw based rations. *Indian Journal of Animal Nutrition*, **10**: 21-25.
- Reddy, D.V. (1997). The effect of supplementation of legume straws on utilization of rice straw-poultry droppings-rice bran-fish meal based diet in buffaloes. *Animal Feed Science and Technology*, **69**: 305-314.
- Schingoethe, D. J., Buyers, F.M. and Schelling, G.T. (1988). Nutrient needs during critical periods of the life cycle. The Ruminant Animal: Digestive Physiology and Nutrition. Ed. D. C. Church. New Jersey: Prentice-Hall, Inc. 421-447.
- Senthilkumar, S., Valli, C. and Balakrishnan, V. (2007). Evolving specific non starch polysaccharide enzyme mix to paddy straw for enhancing its nutritive value. *Livestock Research for Rural Development*, **19** (12): 178. <http://www.cipav.org.co/lrrd/lrrd19/12/sent19178.htm>
- Senthilkumar, S., Viswanathan, T.V., Mercy, A.D., Gangadevi, P., Ally, K. and Shyama, K. (2010). Chemical composition of brewery waste. *Tamilnadu Journal Veterinary and Animal Sciences*, **6**(1) 49-51.
- Singh, H., Sahu, D.S. and Agarwal, I.S. (2001). Effect of feeding urea ammoniated rice straw with varying level of cotton seed cake on the performance of cross bred heifers. *Indian Journal of Animal Sciences*, **71**: 465-467.
- Singh, R., Sharma, S.D., Singh, S.K. and Agrawal, I.S. (2002). Effect of supplementation of lime treated paddy straw with phosphoric acid on rumen metabolism and nutrient utilization in crossbred heifer. *Indian Journal of Animal Nutrition*, **19**: 7-13.
- Sliwinski, B.J., Soliva, C.R., Machmüller, A. and Kreuzer, M. (2002). Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation *Animal Feed Science and Technology*, **101**: 101-114.
- Snedecor, G.W. and Cochran, W.G. (1994). *Statistical Methods*. Eighth edition. The Iowa state University press, Ames, Iowa, pp. 313.
- Sohane, R.K. and Singh, M. (2001). Effect of supplementation green forages with straws from two rice varieties on nutrient utilization in cattle. *Indian Journal of Animal Nutrition*, **18**: 1-7.
- Van Soest, P. J. (1982). Nutritional ecology of the ruminant, 2nd ed. Ithaca, NY: Cornell University Press.
- Vansoest, P.J. and Jones, L.H.P. (1968). Effect of silica in forages upon digestibility. *Journal of Dairy Science*, **51**: 1644-1648.
- Wanapat, M. (1984). Improving rice straw quality as ruminant feed by urea treatment in Thailand. In: Proceeding of the International workshop on relevance of crop residues as animal feeds in developing countries (Ed.M.Wanapat and C.Devendra) Khon Kaer University, Thailand, Nov. 29-Dec. 2, pp. 147-175.