USE OF LIGNOCELLULOLYTIC MUTANTS OF *PLEUROTUS OSTREATUS* IN RUMINANT FEED FORMULATIONS

Vijaya Chalamcherla,[a] Singaracharya Maringanti A,[a*] Vijaya Lakshmi Muvva,[b] Lakshmi Narasu Mangamoori,[c] and Mallikarjuna Reddy Ramireddy [d]

Two lignolytic mutants (POM_1 - U.V. irradiated and POM_2 – X ray irradiated) of *P. ostreatus* wild type (POW) were developed and used in new feed formulations for ruminants. Paddy straw (10 kg) amended with coconut cake, glyricidia leaves, urea (2%), and rice bran (5%) along with mutant forms of *P. ostreatus* substantially increased the reducing sugars, crude protein, and I*n Vitro* Dry Matter Digestibility (IVDMD), while reducing the lignin contents. Maximum amounts of reducing sugars (555 mg/100 ml) were reported in ration 1 with strain POW in 10 days of incubation period, while a minimum was recorded in POM_1 (380 mg / 100 ml) in ration 1 in 30 days of incubation. U.V. irradiated mutant was responsible for accumulation of high crude protein (CP : 42.1 mg / 100 g) in ration 1 after 30 days of incubation. The percent lignin loss was more by mutant forms, and this loss was increased with increased incubation. As a consequence, IVDMD% has been gradually improved and maximized with feed formulations 3 (49.71%) and 1 (47.07%).

Key Words: Rations; Feed formulations; Wild and mutant forms; P. ostreatus.

Contact information: a: Department of Biotechnology, S.V.U.P.G.Centre, Kavali, Andhra Pradesh INDIA. a: Department of Microbiology, Kakatiya University, Warangal, Andhra Pradesh INDIA . b: Department of Microbiology, A.N.U., Guntur, Andhra Pradesh. INDIA.. c: Department of Biotechnology, J.N.T.U., Hydrerabad, Andhra Pradesh. INDIA d: Department of Biotechnology, Jawahar Bharathi Degree and P.G.Studies, Kavali, Andhra Pradesh. INDIA. [a]Corresponding author: E-Mail: vijayalch@gmail.com*

INTRODUCTION

Livestock production is a major component of the agricultural economy of developing countries like India and goes well beyond direct food production. This is closely linked to the social and cultural lives of millions of resource-poor farmers for whom animal ownership ensures a varying degree of sustainable farming and economic stability. However, ruminant production in mixed farming has lagged behind in productivity because of factors such as low genetic potential, poor health management, and imbalanced feeding (Singh et al. 1997). There exists a huge gap between the availability and requirement of feed resources in developing countries like India. The availability of green fodder is 30% and concentrates is 43% lower than the requirements, while the availability of crop residues or dry fodder is 22% higher than their requirement (Garg et al 2007). In this context of feed scarcity also, it is necessary to enrich the crop residues in terms of both digestibility and protein content. The major limitation with crop residues like paddy straw includes their bulkiness and poor nutritive value. Unfortunately, there has been relatively little acceptance by farmers regarding various enrichment (treatment) technologies (physical, chemical and biological), available to

improve the nutritive value (Owen and Jayasuriya 1989; Dolberg 1992; Devendra 1997). Thus, the only viable option left is to concentrate on strategic supplementation with other feeds that provide additional protein, minerals, and energy. Of the several alternative supplementation strategies that may be adopted, the most common is the use of protein supplements, such as oil cake (Devendra 1997). However the choice of oil cake by resource poor farmers is usually limited due to its availability and cost.

Considerable research (Hall 2002; Schwab et al. 2002; Hinders 1998) has gone into identifying and assessing the various possibilities of strategic supplementation with leguminous tree leaves, cassava leaf meal (high fibre high protein), oil seed cakes (coconut, groundnut, sesame, cotton seed), and brans (rice, wheat, maize), which have low fiber and high protein contents. Devendra (1997) and Sharma et al. (2006) evaluated the nutritive value and biodigestability of many novel feed formulations supplemented with edible mushrooms, oil cake, and leguminous tree leaves. However, all of these strategies were worked out in pilot scale in *in vitro* conditions and evaluated on farm testing and demonstration for further documentation of the effect of pre-treatments and supplementation (Doyle et al. 1986). More research is required in on-farm feeding trials involving the physiological and other effects of supplementing fibrous feeds (Steinbach 1997). The present study is oriented to develop efficient new feed formulations for ruminants by amending the paddy straw with cheap, easily available substrates, such as coconut cake, groundnut cake, cotton seed cake, sunflower cake, gliricidia / leucaena / groundnut / erythring / cowpea leaves, and rice bran, etc. inoculated with the mutated forms of *P. ostreatus*, which is very popular as an edible mushroom and considered as a single cell protein in solid state fermentations.

EXPERIMENTAL PROCEDURES

Microorganism and Mutagenesis

The *Pleurotus ostreatus* was collected from the nearby forest areas, cultured, purified, identified, and preserved in KU culture collection centre (Ac No. 207). Mutant strains of *Pleurotus ostreatus* were obtained with the objective of enhancing their lignolytic activity. For the development of mutants, actively growing *P. ostreatus* was inoculated in to the central part of the sterile malt agar plates and incubated for 3 days at 37^{o}C. After sufficient growth, the plates were exposed to UV. rays and X-rays. In the UV irradiation, the plates were exposed for 10, 20, 30, 40, 50, and 60 minutes at an intensity of 83µ Wcm^{-2}. During X-ray irradiation, the plates were exposed for 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 seconds at 40 mA and 70 KV. Unexposed plates served as control (Vijaya et al. 2006). After the exposure, all the plates including control were further incubated at 37^{o}C for 4 days and approximately 2mm^{2} of the fungal mycelium from the spreading edge of each plate was sub-cultured into a separate sterile malt agar slant. All the experiments were conducted in triplicate. The mutants were shown to have potential in selective delignification (Vijaya et al. 2006).

Feed Formulations / Rations

Based on calculation of demand and supply of nutrients for ruminants and in the view of providing balanced diet, different feed formulations with locally available feed substrate were prepared and subjected for SSF using *P. ostreatus*.

Lignocellulosic basal feed - paddy straw (10 kg) was chopped (2 cm long) and soaked overnight in hot water, air dried, and used as a basal fodder for different rations, as shown in Table 1. Other nutrient supplements are taken in different proportions and combinations in different rations.

Table 1. Recipes for Rations Considered in this Work

Feed formulation or Ration	Basal feed Rice straw (Kg)	Low fibre and high protein feed (1 Kg)	High fiber, High protein feed (2 Kg)	N_2 supplementation (Urea)	High DMD* feed Rice bran (g)
R_1	10	Coconut cake	Glyricidia leaves meal	2%	500 g
R_2	10	Groundnut cake	Leucaena leaves	2%	500 g
R_3	10	Sesame cake	Groundnut leaves	2%	500 g
R_4	10	Cotton seed cake	Erythring leaves	2%	500 g
R_5	10	Sunflower cake	Cowpea leaves	2%	500 g

*DMD – Dry Matter Digestibility

Solid State Fermentation

All the above ingredients were mixed properly, and to this mixture a shake culture of *Pleurotous ostreatus* (7 days old), was added (2 liters), remixed, distributed into different conical flasks (1 liter capacity), and incubated for 10, 20, and 30 days at room temperature. Each ration was prepared in quadruples of which 3 sets were used for inoculation of

a) *Pleurotus ostreatus* (wild type) (POW)

b) *P. ostreatus* – U.V irradiated mutant (POM_1)

c) *P. ostreatus* – X-ray irradiated mutant (POM_2)

The fourth set without inoculation of fungi served as control (0^{th} day of incubation). The same procedure was followed for all rations and allowed for solid state fermentation with 70% moisture content in the flasks. During solid state fermentation of these feed formulations a temperature of 37^oC and RH 30% was maintained. Throughout the incubation, periodical shaking of the flasks was performed. After appropriate periods of incubation (10, 20, and 30 days) from each set 100 g of contents were taken, air-dried, ground in 100 ml distilled water, centrifuged, and filtered. Different parameters such as

the amount of reducing sugars, percent saccharification, crude protein, lignin loss, and *in vitro* dry matter digestibility, were analyzed according to the standard procedures (Jageshwar 1940; AOAC 1980; Mehrez and Orskov 1977; Miller 1959; Plummer 1993). All the experiments were conducted in triplicate, and the data obtained was subjected to statistical analysis (2-factor ANOVA-Analysis of Varians). Standard deviation, percentage deviation over control, and F values were calculated and interpreted accordingly. If the calculated F value of the treatment / subject exceeds the F critical value then the effect of treatment / subject is considered as significant.

RESULTS AND DISCUSSION

Changes in Reducing Sugars and Percent Saccharification

The results of all the experiments were analyzed with reference to the efficiency of the organism used, optimum time of incubation, and desirable ration. The amount of reducing sugars and saccharification (%) with new feed formulations during 10, 20, and 30 days of incubation period was analyzed in *P. ostreatus* wild (POW), UV mutant (POM_1), and X-ray mutant (POM_2), and is presented in Table 2. From the table it is evident that the amount of reducing sugars and saccharification (%) increased substantially over the control in the new feed formulations (rations 1 to 5). However, by the addition of glyricidia leaves, rice bran, coconut cake, sunflower cake, etc., the reducing sugars and saccharification (%) increased by several multiples. Maximum amounts of reducing sugars (563 mg/ 100 ml) were reported for ration 1 with strain POW after 10 days of incubation period, while the minimum was recorded in POM_1 (388 mg / 100 ml) for ration 1 after 30 days of incubation time. The results were analysed statistically (Two factor ANOVA) and it was observed that the change in the amount of the reducing sugars and saccharification percentage of different rations over different incubations was insignificant. But SSF with wild and mutant forms showed significant variations in these two parameters (Table. 2A). At the initial incubations all the strains were responsible for maximum reducing sugars, but their subsequent incubations of 20 and 30 days showed gradual decrease. This fall of values of sugars after 10 days might be due to reutilization of sugars by organism for its own growth, as during the early stages of fungal growth lignin removal is more selective. Similar to the present investigation Kaur et al. (1988) also demonstrated the reduction in proportion of reducing sugars after 7 days of SSF of lignocellulosics by *P. ostreatus*. This could be explained by a fact that some fungi, i.e. selective delignifiers, utilize xylan and not cellulose as a co-substrate for the degradation of lignin under *in vitro* conditions. Thus, there is less amount of glucose and other reducing sugars in the hydrolysate (Blanchette et al. 1985).

The results clearly indicated that the supplementation with coconut cake and glyricidia leaves was responsible for the release of high amounts of reducing sugars and saccharification (%). Thus the increase in the quantity of reducing sugars and percentage of saccharification is the desirable change indicating the efficiency of biological treatment ($P<0.001$).

Table. 2. Amount of Reducing Sugars and % Saccharification of Feed Formulations after 0, 10, 20, and 30 days of SSF by POW, POM_1 and POM_2.

Ration	Reducing Sugars (mg/100 ml)									
	A	PO W			PO M_1			PO M_2		
		B	C	D	B	C	D	B	C	D
R_1	390 ±0.90	563 ±0.78	509 ±0.88	456 ±0.81	453 ±1.13	408 ±0.86	388 ±0.58	470 ±0.65	428 ±1.28	405 ±0.67
R_2	370 ±0.59	504 ±0.63	480 ±0.57	459 ±0.74	490 ±0.98	476 ±0.74	441 ±0.69	500 ±0.68	479 ±1.31	455 ±0.99
R_3	320 ±0.56	533 ±0.71	528 ±0.68	506 ±0.85	496 ±0.93	486 ±0.85	471 ±0.74	518 ±0.95	498 ±0.92	478 ±1.26
R_4	380 ±0.72	548 ±0.55	538 ±0.61	507 ±1.23	508 ±0.87	496 ±0.96	470 ±0.84	520 ±0.89	498 ±0.83	483 ±1.38
R_5	375 ±0.82	528 ±0.58	513 ±0.53	507 ±1.15	518 ±1.41	508 ±1.47	490 ±0.59	528 ±1.35	528 ±0.71	488 ±1.46

Ration	% Saccharification									
	A	PO W			PO M_1			PO M_2		
		B	C	D	B	C	D	B	C	D
R_1	351 ±0.90	506.7 ±0.78	458.1 ±0.88	410.4 ±0.81	407.7 ±1.13	367.2 ±0.86	349.2 ±0.58	423 ±0.65	385.2 ±1.28	364.5 ±0.67
R_2	333 ±0.59	453.6 ±0.63	432 ±0.57	413.1 ±0.74	441 ±0.98	428.4 ±0.74	396.9 ±0.69	450 ±0.68	431.1 ±1.31	409.5 ±0.99
R_3	288 ±0.56	479.7 ±0.71	475.2 ±0.68	455.4 ±0.85	446.4 ±0.93	437.4 ±0.85	423.9 ±0.74	466.2 ±0.95	448.2 ±0.92	430.2 ±1.26
R_4	380 ±0.72	493.2 ±0.55	484.2 ±0.61	456.3 ±1.23	457.2 ±0.87	446.4 ±0.96	423 ±0.84	468 ±0.89	448.2 ±0.83	434.7 ±1.38
R_5	342 ±0.82	475.2 ±0.58	461.7 ±0.53	456.3 ±1.15	466.2 ±1.41	457.2 ±1.47	441 ±0.59	475.2 ±1.35	475.2 ±0.71	439.2 ±1.46

$$\% \text{ saccharification} = \frac{\text{Amount of reducing sugars (0.9)}}{\text{Amount of substrate}} \times 100$$

POW - *P. ostreatus* wild type
POM_1 - *P. ostreatus* U.V.irradiated mutant
POM_2 - *P. ostreatus* X-ray irradiated mutant

A – 0 days incubation (Control)
B - 10 days incubation
C - 20 days incubation
D - 30 days incubation

All values are Statistically Significant at P<0.001
All values are Mean ± SD of three individual observations.
PDC* : Percent deviation over control.

Table. 2 A. ANOVA Results for Changes in Reducing Sugars of Rations after 10, 20, and 30 Days of Incubation Successively

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	Result
Rations	2117.3	4	529.325	0.853027	0.5187557	3.2591667	Insignificant
Strains	83237.2	3	27745.73	44.71332	8.667E-07	3.4902948	Significant
Error	7446.3	12	620.525				
Total	92800.8	19					

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Result*
Rations	6135.3	4	1533.825	1.769203	0.199843	3.259167	Insignificant
Strains	62134	3	20711.33	23.88965	2.39E-05	3.490295	Significant
Error	10403.5	12	866.9583				
Total	78672.8	19					

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Result*
Rations	8046.7	4	2011.675	2.725816	0.079777	3.259167	Insignificant
Strains	40710.15	3	13570.05	18.38739	8.78E-05	3.490295	Significant
Error	8856.1	12	738.0083				
Total	57612.95	19					

Table. 2 B. ANOVA Results for Changes in % Saccharification of Rations after 10, 20, and 30 days of Incubation Successively

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Result*
Rations	3001.688	4	750.422	1.323918	0.316475	3.259167	Insignificant
Strains	59385.17	3	19795.06	34.92306	3.3E-06	3.490295	Significant
Error	6801.828	12	566.819				
Total	69188.69	19					

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Result*
Rations	6616.043	4	1654.011	2.245928	0.124828	3.259167	Insignificant
Strains	43454.38	3	14484.79	19.66843	6.32E-05	3.490295	Significant
Error	8837.385	12	736.4488				
Total	58907.81	19					

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Result*
Rations	8214.902	4	2053.7255	3.271407	0.049484	3.259166	Significant
Strains	27512.484	3	9170.828	14.60833	0.000261	3.490294	Significant
Error	7533.366	12	627.7805				
Total	43260.752	19					

Changes in Crude Protein Content of Feed Formulations

The increase in crude protein (CP) of all these feeds after 0, 10, 20, and 30th days of SSF by wild and mutant strains of *P. ostreatus* were analyzed and are presented in Figs. 1 through 3. From these figures it was noted that the crude protein content was further improved by 4 - 8 (60%) fold in feed formulations amended with oil cake, legume leaves, and brans. There was further increase in crude protein values with increased incubation periods for all rations with the modified strains. U.V. irradiated mutant (POM_1) was responsible for accumulation of high crude protein (42.1 mg / 100 g) on the 30th day of inoculation in ration 1. Among five feed formulations, 1 and 3 were with more amount of crude protein, i.e. 42.1 mg / 100g and 40.6 mg / 100 g respectively and the least crude protein content (33.3 mg / 100 g) was noticed in POM_2 on the 10th day of incubation. The statistical analysis also indicated much more significant change in the CP values of different rations (F: 3.2947) and also with different strains (F: 362.5934).

According to Hatakka (1989) agricultural lignocellulosic materials enriched by fungal protein may be a valuable by-product if wood rotting fungi are used for the production of extracellular enzymes. Further, the nutritive value investigated with the rats, pigs, and sheep showed that fungal protein is more suitable for ruminants than for mono gastric animals. This is probable due to the cell-wall structure of the fungus. The digestibility of the fungal protein by sheep is 82% (Thomke et al 1980). In this context the increase in CP value by 4-8 folds is considered as suitable and is a desirable sign in improvement of the nutritional status of feed formulations. The statistical analysis is also clearly indicating the significance.

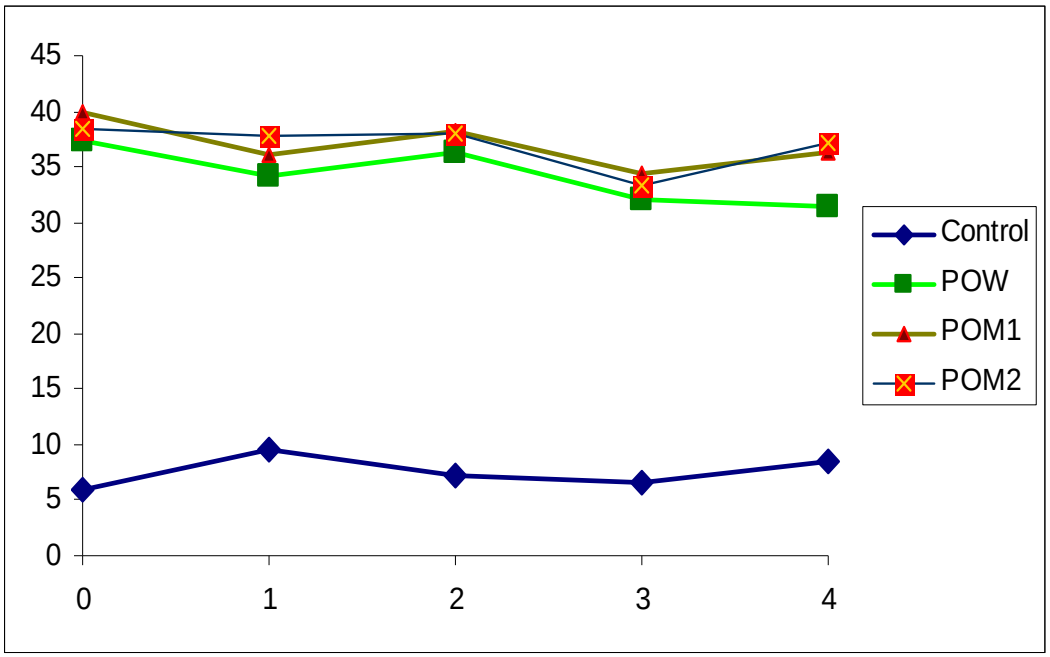


Fig. 1. Increase in CP values of feed formulations after 10 days of SSF by POW, POM_1, and POM_2

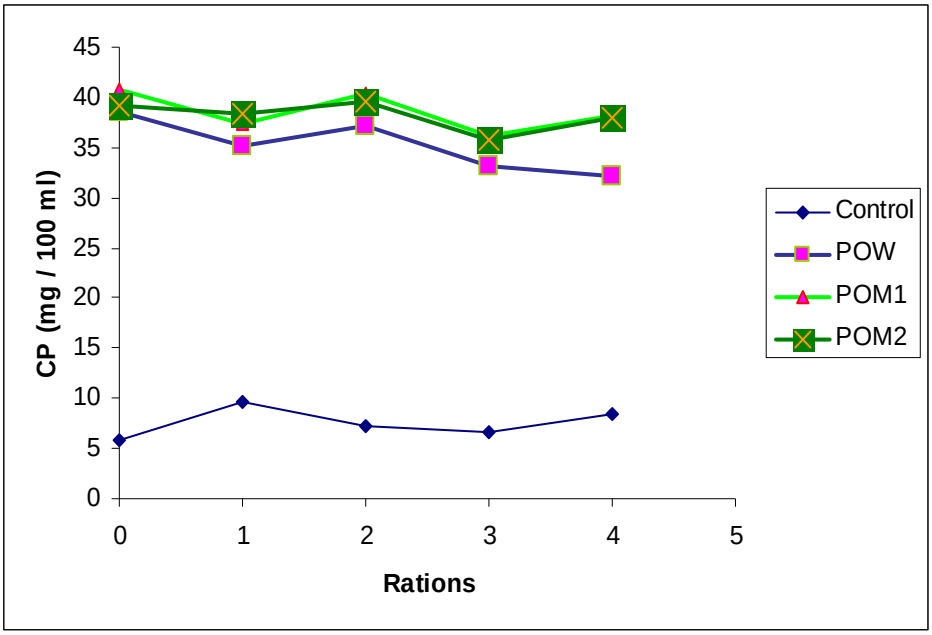


Fig. 2. Increase in CP values of Feed formulations after 20 days of SSF by POW, POM_1, and POM_2

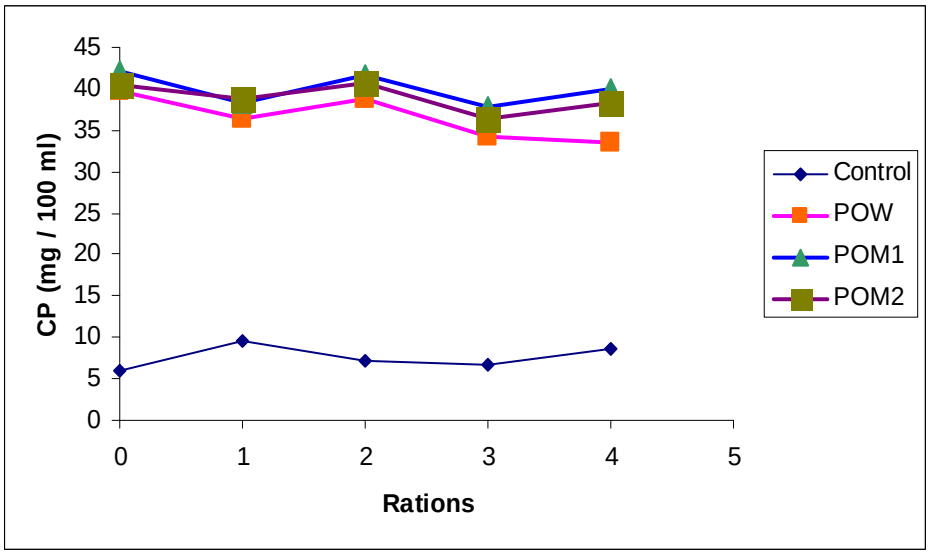


Fig. 3. Increase in CP values of Feed formulations after 30 days of SSF by POW, POM_1, and POM_2

Rations: 0 - R_1, 1 - R_2, 2 - R_3, 3 - R_4, 4 - R_5

POW - *P. ostreatus* wild type
POM_1 - *P. ostreatus* U.V.irradiated mutant
POM_2 - *P. ostreatus* X-ray irradiated mutant

A – 0 days incubation (Control)
B - 10 days incubation
C - 20 days incubation
D - 30 days incubation

Changes in Percent Lignin Loss and IVDMD

During SSF of modified feed formulations by wild and mutant forms of *P. ostreatus*, the percent lignin loss and changes in *in vitro* Dry Matter Digestibility were measured after 0, 10, 20, and 30 days of incubations and are presented in Figs. 4 and 5. From these figures it was evident that percent lignin loss was more by mutant forms than by wild *P. ostreatus*. Lignin content was gradually reduced with increased incubations. As a consequence, IVDMD% has been improved gradually with increased incubations, and high IVDMD% was noted (51.75%) by POM_1 in feed formulation 3 on its 30th day of incubation. POM_1 (U.V. mutant) was found to be more efficient than the X-ray mutant and wild varieties in removing lignin effectively with all feed formulations. However, both these mutants accounted for 80-90% more lignin loss in all feed rations than the wild type. The rate of lignin degradation was maximum with POM_1 in ration 3 on its 30th day of incubation (42.1%). Among all the feeds tested the percent lignin loss was maximum in ration 3, and minimum in rations 4 and 5. Further, it was clearly shown that in POW, there was 0.6 to 0.7% loss of lignin per day, but it was 1.2 to 1.4% per day with mutant strains. *In vitro* dry matter digestibility was improved remarkably and was maximum with ration 3 (51.75 %) by POM_1 after 30 days of SSF, and these values were minimum with ration 2, inoculated by POW after 30 days of SSF (36.11%). Lignin degradation started in earlier incubation periods by mutants than wild type and reached a maximum in 30 days of SSF. Further, these results were analysed statistically and it was observed that the percent lignin loss and IVDMD changes were more significant in all rations over different incubations (F- calculated value 5.3952 is less than F- critical value 3.2591). The stastistical analysis emphasizes that the SSF of different feed formulations using mutant forms of *P. ostreatus* improves the lignin loss significantly compared to *P. osreatus* wild strains.

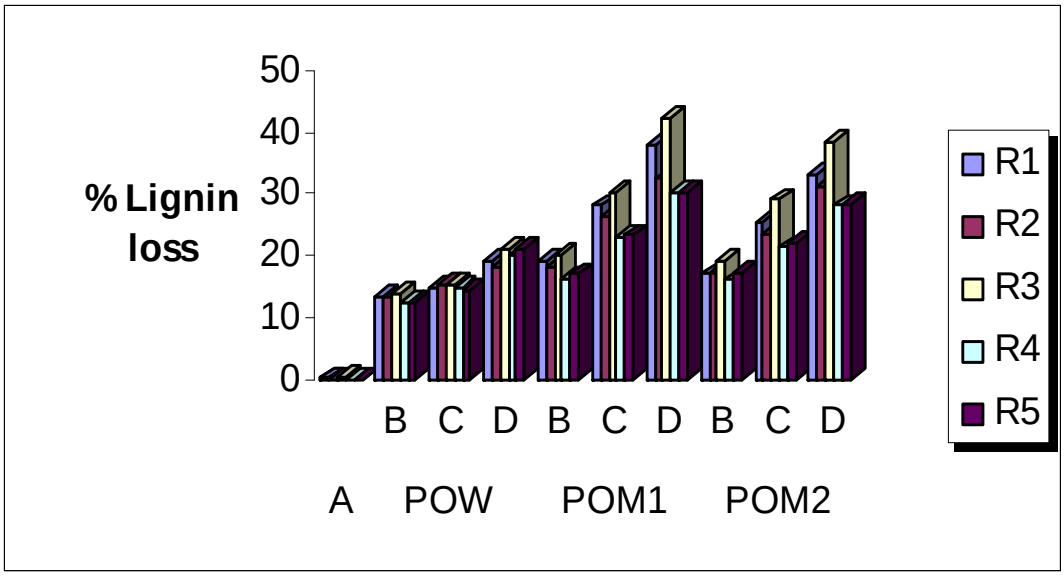
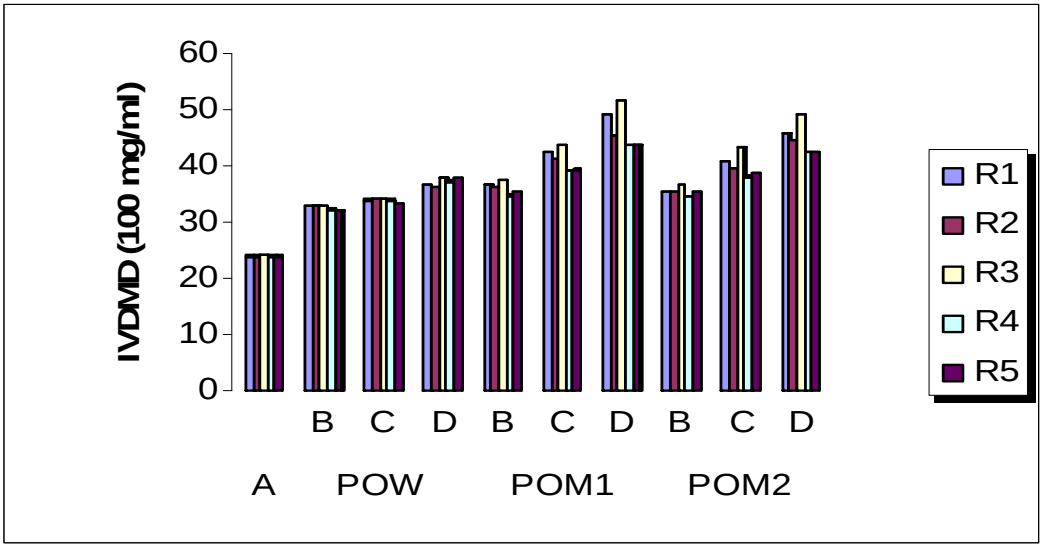


Fig. 4. Lignin loss (%) in five rations after 10, 20, and 30 days of SSF by POW, POM1, and POM2



IVDMD% = 23.97+0.66 (%loss of lignin)

Fig. 5. *In vitro* dry matter digestibility (IVDMD) (%) in five rations after 10, 20, and 30 days of SSF by POW, POM_1, POM_2

POW - *P. ostreatus* wild type
POM_1 - *P. ostreatus* U.V. irradiated mutant
POM_2 - *P. ostreatus* X-ray irradiated mutant

A – Control (0 days)
B - 10 days of incubation
C - 20 days of incubation
D - 30 days of incubation

Singh and Srivatsava (1990) observed that the increase in IVDMD in all the strains tested was due to higher dry matter loss during the process of treatment. Similarly, there was a substantial improvement in the IVDMD of paddy straw with variety of combinations in mutant forms over the control. Similar to the present data, the investigations of Zadrazil and Kamra (1989) also demonstrated the improvement in *in vitro* digestibility of wheat straw after fermentation with *Pleurotus sp*. and *Stropharia rugusoannulata*. Degradation of lignin to water soluble compounds is more important for enhancement of digestiblitiy than its degradation to carbon dioxide (Blanchette 1984). The increased (lignin decomposition) delignification by mutants (POM_1 and POM_2) consequently leads to increase in IVDMD, which is considered as a positive change in increasing the overall digestibility of feed formulations. Boguhn et al. (2006) worked to identify dietary factors that affect fermentation and efficiency of microbial protein, and fermentation of different fractions was assessed, where organic matter was raised from 35-47%, crude proteins 25-60%, neutral detergent fiber 3-28%, and gross energy range was 31-45%. Jung et al. (1992) assessed the effect of five white-rot basidiomycetes on chemical composition and *in vitro* digestibility of straws for 30 days at 28^{o}C and 90% RH and noticed that cell-wall polysaccharides were removed from the straw, while IVDMD decreased because the fungi removed the most readily fermentable polysaccharides.

Salmones et al. (2005) studied the comparative culturing of *Pleurotus* species on coffee pulp and wheat straw and reported the degradation of polysaccharide compounds with the fruiting stage, while the reduction in phenolic contents was detected in both substrates during the first eight days of incubation. Tripathi et al. (2008), during their selection of white-rot basidiomycetes for bioconversion of mustard (*Brassica compestris*) straw under SSF into energy substrate for rumen microorganisms, found that of the three fungi tested (*Phanerochaete chrysosoporium*, *Ganoderma applanatum*, and *Coriolus versicolor*), the IVDMD and CP contents were higher in *C. versicolor* cultured straw with higher delignification between 7 and 28 days of fermentation.

CONCLUSIONS

In view of these observations and perception of the farmers an appropriate feed formulation was developed, suitable for poor farmers of this region.

1. The availability of feed materials such as oil cake, legume tree leaves, bran etc. and the fermentation with developed lignocellulolytic mutant strains of *Pleurotus ostreatus* was considered.

2. Maximum amount of reducing sugars and saccharification percentage was noted with ration 1.

3. UV irradiated mutant (POM_1) was responsible for accumulation of high crude protein. The percent lignin loss was more with mutant forms with increased incubation. IVDMD % was maximum with feed formulation 3.

4. Thus in the present study among all the feed formulations tried, formulation 3 composed of paddy straw, rice bran, 2% urea, *Erythring* leaves, and sesame cake inoculated with *P. ostreatus* U.V. mutant and subjected to 30 days of SSF was found to be a suitable and optimum feed for ruminants, followed by ration 1 supplemented with coconut cake and *Glyrecida* leaves.

REFERENCES CITED

AOAC (1980). "Official methods of analysis," 14th Ed. *Association of Official Analytical Chemists*, Washington. D.C.

Beltran, P. C., Carniti, P., Visciglio, A., Focher, B., and Marzethi, A. (1992). "Fractionation and bioconversion of steam exploded wheat straw," *Bioresource Technol*. 39, 165-171.

Blanchette, R. A., Otjen, L., Effland, M. J., and Eslyn, W. E. (1985) "Changes in structural and chemical components of wood delignified by fungi," *Wood Science Technol*. 19, 35-46.

Boguhn, J., Kluth, H., and Rodehuts Cord, M. (2006). "Effect of total mixed ration composition on fermentation and efficiency of ruminal microbial crude protein synthesis *in vitro*," *J. Dairy Sci*. 89, 1580-1591.

Devendra, C. (1997). "Crop residues for feeding animals in Asia: Technology development and adoption in crop / livestock systems," In *Crop Residues in Sustainable Mixed Crop / Livestock Farming Systems* (Ed. Renard), CAB International, Wallingford., 241 – 267.

Dolberg, F. (1992). "Progress in the utilization of urea ammonia treated crop residues: Biological and Socio economic aspects of animal production and application of the technology on small farms," *Livestock Research for Rural Development.* 4, 20-31.

Doyle, P. T., Devendra, C., and Pearce, G. R. (1986). "Rice straw as a feed for ruminants," International Development Programme of Australian Universities and Colleges (IDP), Canberra, Australia.

. Garg, M. R., Sherasia, P. C., and Bhanderi, B. M. (2007). "Slow ammonia release and protected protein supplement to economize milk production in dairy animals," *Indian Dairyman* 59(12), 43-49.

Hall, M. B. (2002). "Ruminant nutrition; New concepts and development in forage and feedstuff analysis and applications to ruminant nutrition: Characterizing carbohydrates in feeds," *J. Anim. Sci*. 80, 146.

Hatakka, A. I. (1983). "Pretreatment of wheat straw by white–rot fungi does enzymic saccharification of cellulose," Eur. *J. Appl. Microbiol. Biotechno*l. 18, 350-357.

Hatakka, A. I., Mohammdi, O. K., and Lundell, T. K.(1989). "The potential white rot fungi and their enzymes in the treatment of lignocellulosic feeds," *Food Biotech*. 3, 45-58.

Hinders, R. G., (1998). "Use of new concepts in feed formulation and feeding for high producing cows," *J. Dairy Sci*. 85, 146-147.

Jageshwar, G. S. (1940). "Estimation of lignin in tannin materials," *Biochem. J*. 50, 783-789.

Jung, H. G., Valdez, F. R., Abad, A. R., Blanchette, R. A., and Hatfield, R. D. (1992). "Effect of white rot basidomycetes on chemical composition and *in vitro* digestibility of oat straw and alfa alfa stems," *J. Anim. Sci*. 70, 1928-1935.

Kaur, A., Dhillon, R., Aggarwal, N., and Kahlon, R. S. (1998). "Solid state fermentation of lignocelulosics by *Pleurotus ostreatus*," In: *Fungi in Biotechnology.* (Ed. Anil Prakash). CBS Publishers, New Delhi., p. 187-191.

Mehrez, A. Z., and Orskov, E. R. (1977). "A study of the artificial fiber-bag technique for determing the digestibility of feeds in the rumen," *J. Agr. Sci*. 88, 645-650.

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for the determination of reducing sugars," *Analyt. Chem*. 31, 426-428.

Owen, E., and Jayasuriya, M. C. M. (1989). "Use of crop residues as animal feeds in developing countries: A review," *Research Development in Agriculture* 6, 124-128.

Plummer, D. T. (1993). *An Introduction to Practical Biochemistry*, Tata McCraw-Hill, Pub. New Delhi., 180-181.

Salmones, D., Mata, G., and Waliszewski, K. N. (2005). "Comparative culturing of *Pleurotus* sp. on coffee pulp and wheat straw: Biomass production and substrate biodegradation," *Bioresour Technol*. 96, 537-544.

Schwab, C. G., Tylutki, T. P., Sheaffer, C., and Stern, M. D. (2002). "Ruminant nutrition; New concepts and development in forage and feedstuff analysis and applications to ruminant nutrition: Characterizing proteins in feeds," *J. Anim. Sci*. 80, 146.

Sharma, K., Dutta, N., and Naulia, U. (2006). "Livestock research for rural development. Nutritional and socio-economic implications of feeding cereal straws supplemented with higher dietary level of de-oiled cakes to lactating buffaloes (*Bubalus bubalis*): An on-farm study,"

Singh, K., Habib, G., Siddiqui, M. M., and Ibrahim, M. N. M. (1997). "Dynamics of feed resources in mixed farming systems of South Asia," in C. Renard (ed.) *Crop Residues in Sustainable Mixed Crop / Livestock Farming Systems* (CAB International, Wallingford)., 113 - 130.

Singh, R., and Srivastava, S. K. (1990). *J. Microbial Biotechnol*. 5, 1-11. *

Steinbach, J. (1997). "Alternatives to crop residues as feed resources in mixed farming Systems" in C. Renard (ed.), *Crop Residues in Sustainable Mixed Crop / Livestock Farming Systems* (CAB International, Walling ford)., 197-214.

Thomke, S., Rundgren, M., and Eriksson, S. (1980). "Nutritional evaluation of the white-rot fungus *Sporotrichum pulverlentum* as a feedstuff to rats, pigs and sheep," *Biotechnol. Bioeng*. 22, 2285-2303.

Tripathi, M. K., Mishra, A. S., Mishra, A. K., Vaithiyanathan, S., Prasad, R., and Jakhmola, R. C. (2008). "Selection of white-rot basidiomycetes for bioconversion of mustard (*Brassica compestris*) straw under solid-state fermentation into energy substrate for rumen microorganism," *Letters in Applied Microbiology* 46, 364-370.

Vijaya, C. H., Singaracharya, M. A., and Vijaya lakshmi, M. (2006). Selection of cellulase minus white rot fungi for biobleaching and biopulping," *National J. Life Sciences* 2, 133-138.

* Originals not consulted in one case.

Article submitted: July 11, 2008; Peer review completed: Sept. 12, 2008; Revised version received and accepted: Dec. 3, 2008; Published: Dec. 9, 2008