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Effect of Urea, Biological Inoculant, Molasses and Fiber Degrading Enzymes on *in vitro* Ruminal Fermentation of Paddy Straw Silage

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ABSTRACT

This study was planned to ascertain the effect of inclusion of urea, biological inoculant, fiber degrading enzymes and molasses on chemical composition, *in vitro* utilization and degradability of paddy straw silage. Urea was used @0%, 1% and 2%. Molasses (M) was added @0%, 3% and 6%. *Lactobacillus plantarum* (LAB) @ $2.4x10^6$ cfu/g and enzymes (no addition, xylanase or cocktail) @ 50 g/100 kg paddy straw were included in some treatments and excluded in others. The DM, ADL, NDICP and ADICP were found to be significantly higher ($p<0.05$) and NDF, hemicellulose and cellulose showed a significant decline ($p<0.05$) with the inclusion of urea @1% and molasses@6%. *Lactobacillus* inclusion caused a significant decline in NDF, ADF and silica ($p<0.05$). Evaluation of *in vitro* utilization showed significantly higher ($p<0.05$) NGP, ml, OMD, mg, OMD,% , DMD, % , MCP,% and ME, MJ/kg DM with urea@1%, molasses@6% and with the inclusion of *Lactobacillus plantarum* and cocktail enzyme.

HIGHLIGHTS

- Urea@1% and molasses@6% increased DM, ADL. NDICP and ADICP while lowering NDF, Hemicellulose and cellulose significantly.
- Supplementation of urea@1%, molasses@6%, inclusion of *Lactobacillus* and cocktail of enzymes significantly increased *in vitro* utilization**.**

Keywords: Paddy straw, urea, *Lactobacillus*, molasses, silage

Paddy production in India, one of the world's top producers and exporters of rice, averages 152.60 MT (million tons) annually (Bindu and Manan, 2018). In India, a significant agricultural byproduct that is typically given to large animals is paddy straw. However, due to the paddy straw's low nutritional value, high lignified and indigestible material concentration and high levels of anti-nutritional elements including lignin, silicates and oxalates, it is insufficient for maintaining animals (Emam *et al*., 2014).

Paddy straw contains about 4% crude protein (CP), 75% Neutral detergent fiber (NDF), 1900 kcal DE/kg of straw, 54% Acid detergent fiber (ADF),1900 kcal DE/kg of straw, 5-15 % Silica, 35% crude fiber (CF), and 2 % oxalates (Khanday *et al*., 2018). Paddy straw contains 8 to 14 percent silica, which is very high. Although burning rice straw is the quickest method of disposal of straw, it increases environmental pollution by causing an upsurge in greenhouse gases in the environment. It is anticipated to be both practicable and environmentally beneficial to use exogenous enzymes and bacterial cultures, such as lactic acid bacteria (*Lactobacillus*), to increase the nutritional value and digestibility of paddy straw. The usage of urea acts as an additive employed to cause an increment in the

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protein content. Molasses additions are used as stimulants to increase the availability of fermentable carbohydrates and to accelerate the growth of LAB in order to improve fermentation quality (Li *et al*., 2010). The inoculation of LAB is intended to produce lactic acid to raise the silage's acidity level, leading to lower dry matter losses than would otherwise occur. There are two main purposes for the enzyme additions used in the production of silage. First, they release free sugars from forage plants that are consumed by fermentative microorganisms to produce acetate and lactate, decreasing the pH and conserving the forage. The second purpose is cell wall degradation, which lowers the overall fiber content of the ensiling materials.

MATERIALS AND METHODS

The current research was conducted in the Animal Nutrition laboratory at the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Preparation of silage

In this study, paddy straw that was obtained from the PAU farm in Ludhiana was employed. For better-quality silage, harvested paddy straw was directly gathered from fields and cut into pieces between one and three inches long using a chop cutter. Straw was ensiled in a plastic container and kept in the BOD incubator for 45 days. 250gm of the chopped substrate was used for each treatment wherein 54 containers were utilized to maintain anaerobic condition and prepare silage in the lab by treatment with urea; at concentrations of 0, 1, and 2 percent, molasses; at concentrations of 0, 3 and 6 percent, and moisture levels of 40 percent with or without xylanase and Cocktail of enzymes which consisted of cellulase and hemicellulase and with or without *Lactobacillus plantarum* culture. Consequently, there were 54 treatments in total (3x3x2x3).

Chemical Analysis

For DM analysis, specified quantity of the sample was taken and heat dried for 24 hours at 100 ^{o}C and difference in weight was determined. A known weight of sample is ignited at approximately 550 ^{o}C in a muffle furnace to quantify the amount of ash present after all organic material has been oxidized and lost as CO_2. Similarly, other proximate principles like crude protein, ether extract were determined as per procedures set by Association of Official Analytical Chemists (AOAC, 2007). Cell wall constituents like NDF and ADF were determined by fiber determination techniques (Van Soest *et al*., 1991). The difference between NDF, ADF and ADL was used to compute the hemicellulose and cellulose concentrations.

In vitro evaluation

The *in vitro* gas production analysis was done according to the method devised by Menke and Steingass (1988). After 24 hours, volume of gas produced in each treatment was determined. The following solutions were employed and incubated in a waterbath at 39°C: macro mineral solution, buffer solution, micro mineral solution, resazurine, reducing solution, and rumen fluid obtained from a fistulated male donor. The amount of gas produced after 24 hours was measured in each syringe. The calculation of ME is based on the gas output. Calculated as the ratio of the substrate's true in vitro degradation weight (mg) to the gas volume (ml) it produced, the partition factor (PF) was also determined.

Statistical Analysis

Statistical analysis was performed by using data will be analyzed using SPSS, 2013 software using ANOVA with interaction.

RESULTS AND DISCUSSION

Chemical composition of raw paddy straw

The dry matter (DM) content in paddy straw was 88%. Wanapat *et al*. (2009) reported that paddy straw contained 88.08 % DM which was almost similar to the present study. The crude protein (CP) content of paddy straw was 3.88% and ether extract (EE) was 1.5 %. The NDF and ADF content of paddy straw was 75.6 and 51.5 % respectively and the hemicellulose and cellulose content was 28.9 % and 35.8 % respectively. The chemical composition of paddy straw includes 72.0 % NDF which is nearly similar to the present study. The total ash and AIA content was 15.5 % and 5.25 % respectively. The Acid detergent Lignin (ADL) content was estimated in the present study to be

7.00%. Similarly, the composition of paddy straw reported by Ganai *et al*. (2006) was cellulose 45%, hemicellulose 25-30% and lignin 7-10%.

Effect of different levels of urea on chemical composition and *in vitro* degradability

Urea was used in three graded levels: 0%, 1% and 2%. At 2% urea level, highest crude protein was seen (10.23) while the lowest was evident at 0% urea level (7.64) and it showed significant variation ($p<0.05$). This can be attributed to inclusion of urea at the highest level and exclusion of urea in the first treatment. Ether Extract (1.43), ash (14.56), OM (85.42) did not vary significantly among different levels of urea supplementation. Highest NDF (72.71%), hemicellulose (23.08%) and cellulose (36.11%) were found in paddy straw with no urea supplementation, indicating that inclusion of urea would cause a decline in the relative proportion of these components. NDF, hemicellulose and cellulose varied significantly and was found to be lowest in the treatment with 1% urea. The results obtained are in accordance with a research study conducted by Trach and Tuan (2008) wherein paddy straw was ensiled with urea levels (1, 1.5 and 2%). They found a dramatic increase in crude protein and also noted a significant reduction ($p<0.05$) in NDF with the inclusion

Table 1: Effect of different levels of urea on chemical composition of paddy straw silage

Parameters	0% Urea	1% Urea	2% Urea	p-value	SEM
DM	59.53^{b}	63.056^{a}	59.53^{b}	0.000	0.488
CP	7.64^{c}	8.815^{b}	10.23^{a}	0.000	0.139
EE	1.48	1.422	1.41	0.394	0.023
Ash	14.35	14.46	14.89	0.195	0.157
OM	85.64	85.10	85.53	0.237	0.154
NDF	72.71^{a}	69.95^{b}	68.44^{b}	0.000	0.335
ADF	49.63^{a}	48.89^{a}	46.32^{b}	0.000	0.262
ADL	5.58^{c}	7.18^{a}	6.43^{b}	0.000	0.102
Hemicellulose	23.08^{a}	21.05^{b}	22.12^{ab}	0.015	0.309
Cellulose	36.11^{a}	32.52^{b}	32.20^{b}	0.000	0.353
Silica	8.31^{b}	9.17^{a}	7.94^{b}	0.000	0.124
NDICP	4.75^{b}	5.13^{a}	4.76^{b}	0.000	0.0336
ADICP	4.53^{b}	4.84^{a}	4.50^{b}	0.000	0.0312

Table 2: Effect of different levels of urea on *in vitro* utilization of paddy straw silage

Parameters	Treatments				SEM
	0% Urea	1% Urea	2% Urea	p- value	
NGP, ml	39.58^{b}	41.69^{a}	34.83^{c}	0.000	0.368
NGP, ml/g DM	105.56^{b}	111.17^{a}	92.90^{c}	0.000	0.983
TDS, mg	321.17	318.97	320.74	0.187	0.578
OMD, mg	142.56^{c}	153.45^{a}	147.93^{b}	0.000	1.039
PF, mg/ml	3.62^{c}	4.24^{a}	3.69^{b}	0.000	0.038
OMD,%	44.36^{c}	47.97^{a}	46.18^{b}	0.000	0.278
NDFD,%	34.84	34.82	34.41	0.742	0.331
MMP, mg	55.47^{c}	70.80^{a}	62.211^{b}	0.000	1.070
EMMP, %	38.53^{b}	47.94^{a}	40.34^{b}	0.000	0.585
ME,MJ/kg DM	4.29^{a}	4.49^{a}	4.48^{a}	0.000	0.0985
DMD,%	51.72^{c}	55.14^{a}	53.80^{b}	0.000	0.239
MCP,%	1.40^{c}	1.57^{a}	1.48^{b}	0.000	0.0139
SCFA, mmole	0.464^{b}	0.4894^{a}	0.4082^{c}	0.000	0.004
NH_3-N, mg/dl	29.82	31.25	32.47	0.180	0.566

of urea at 2% level. Evaluation of *in vitro* utilization revealed that with the incorporation of 1% urea, highest levels of NGP, ml (41.69 ml), NGP, ml/g DM (111.17 ml/g DM), OMD, mg (153.93 mg), OMD,% (47.97%), DMD, % (55.14%), MCP,% (1.57%), SCFA, mmole (0.4894 mmole) which varied significantly from other treatments were observed ($p<0.05$).TDS, mg, NDFD,% and NH_3-N, mg/dl did not show any significant difference. PF, mg/ml, MMP, mg and EMMP,% varied significantly when urea was incorporated at the level of 1% (4.24 mg/dl, 70.80 mg and 47.94% respectively). Mesfin and Ledin (2004) worked with urea treated barley straw and reported that urea treatment can improve crude protein (CP) and *in vitro* organic matter digestibility (IVOMD), as well as they found good effects on NDF, ADF content but acid detergent and lignin increased due to urea treatment.

Effect of different levels of molasses on chemical composition and *in vitro* degradability

In the present study, the inclusion of molasses was done in three levels: 0% molasses, 3% molasses and 6% molasses. DM (61.764) and OM (86.14) were found to be significantly higher in 3% molasses inclusion ($p<0.05$). Highest crude protein content (9.29) was found in silage with 6% molasses, intermediate (8.97) in 3% molasses and lowest (8.46) in 0% molasses. Highest fat was seen in 0% molasses (1.52) while lowest was evident with 6%

Table 3: Effect of different levels of molasses on chemical composition of paddy straw silage

Parameters	0% Molasses	3% Molasses	6% Molasses	p- value	SEM
DM	60.16^{ab}	61.764^{a}	58.56^{b}	0.027	0.488
CP	8.46	8.97	9.29	0.050	0.139
EE	1.52^{a}	1.45^{ab}	1.34^{b}	0.016	0.023
Ash	14.65^{ab}	14.08^{b}	15.09^{a}	0.022	0.157
OM	85.35^{ab}	86.14^{a}	84.78^{b}	0.027	0.154
NDF	71.81^{a}	70.82^{a}	68.47^{a}	0.000	0.335
ADF	49.05	47.80	47.99	0.123	0.262
ADL	6.30	6.35	6.55	0.749	0.102
Hemicellulose	22.76^{a}	23.01^{a}	20.48^{b}	0.001	0.309
Cellulose	35.51^{a}	33.06^{b}	32.27^{b}	0.000	0.353
Silica	8.60^{ab}	8.00^{b}	8.82^{a}	0.027	0.124
NDICP	4.87	4.93	4.83	0.538	0.0336
ADICP	4.62	4.65	4.61	0.825	0.0312

Table 4: Effect of different levels of molasses on *in vitro* utilization of paddy straw silage

Parameters	Treatments				
	0% Molasses	3% Molasses	6% Molasses	p -value	SEM
NGP, ml	36.64^{c}	39.00^{b}	40.47^{a}	0.001	0.368
NGP, ml/g DM	97.71^{c}	104.00^{b}	107.92^{a}	0.001	0.983
TDS, mg	320.06^{b}	317.89^{b}	322.93^{a}	0.030	0.578
OMD, mg	144.80	146.67	152.46	0.100	1.039
PF, mg/ml	3.78	3.79	3.98	0.054	0.038
OMD,%	45.81	45.51	47.19	0.216	0.278
NDFD,%	32.88^{b}	35.52^{a}	35.67^{a}	0.002	0.331
MMP, mg	60.87	63.42	64.19	0.322	1.070
EMMP, %	41.21^{b}	41.25^{b}	44.35^{a}	0.033	0.585
ME,MJ/kgDM	4.68^{c}	5.09^{b}	5.50^{a}	0.003	0.0985
DMD,%	53.01	53.43	54.22	0.239	0.239
MCP,%	1.44	1.48	1.54	0.073	0.0139
SCFA, mmole	0.4575^{b}	0.4296^{c}	0.4749^{a}	0.001	0.0043
NH_3-N, mg/dl	28.55^{b}	33.93^{a}	31.07^{ab}	0.001	0.566

molasses (1.34). Highest ash (15.09) and silica (8.82) was seen in 6% molasses while 0% molasses revealed intermediate level and lowest ash (14.08) and silica (8.00) was evident in silage with 6% molasses. Hemicellulose and cellulose content in 6% molasses was the lowest and it varied significantly ($p<0.05$). No significant variation was observed in ADF%, ADL%, NDICP% and ADICP%. Sheikh *et al*. (2017) also had similar results wherein the conclusion of his experiment was that the paddy straw group that had been treated with urea-molasses had significantly ($P<0.05$) greater digestibility of CP, ADF, DM, NDF and cellulose. *In vitro* evaluation revealed that when molasses was added at 6% level, there was significant increase in NGP, ml (40.47 ml), NGP, ml/g DM (107.92 ml/g DM), TDS, mg (322.93 mg), NDFD,% (35.67%), EMMP, % (44.35%), ME,MJ/kg DM (5.50 MJ/kg DM) ($p<0.05$). OMD, mg (152.46 mg), OMD,% (47.19%), DMD, % (54.22%), MCP, % (1.54 %), SCFA, mmole (0.4749 mmole) and NH_3-N, mg/dl (33.93 mg/dl) varied non-significantly from other treatments. The results obtained are in accordance with Zhao *et al*. (2019) who experimented on *in vitro* gas production of paddy straw ensiled with lactic acid bacteria and molasses. They concluded silage with L and ML improved *in vitro* NDFD digestibility when compared to other treatments, while M and ML silage increased *in vitro* neutral detergent fiber degradability (IVNDFD) ($p< 0.05$).

Table 5: Effect of *Lactobacillus* culture on chemical composition of paddy straw silage

Parameters	Without Lactobacillus	With Lactobacillus	p-value	SEM
DM	59.57	60.96	0.230	0.488
CP	8.81	8.98	0.507	0.139
EE	1.40	1.47	0.217	0.023
Ash	14.64	14.50	0.770	0.157
OM	85.35	85.49	0.849	0.154
NDF	71.16[a]	69.57[b]	0.010	0.335
ADF	48.83[a]	47.73[b]	0.045	0.262
ADL	6.36	6.44	0.542	0.102
Hemicellulose	22.33	21.84	0.314	0.309
Cellulose	33.65	33.57	0.876	0.353
Silica	8.84[a]	8.11[b]	0.005	0.124
NDICP	4.85	4.91	0.425	0.0336
ADICP	4.60	4.65	0.427	0.0312

Table 6: Effect of *Lactobacillus* culture on in vitro utilization of paddy straw silage

Parameters	Treatments			
	Without *Lactobacillus*	With *Lactobacillus*	p- value	SEM
NGP, ml	38.43	38.97	0.662	0.368
NGP, ml/g DM	102.49	103.93	0.662	.983
TDS, mg	319.99	320.60	0.775	0.578
OMD, mg	147.06	148.89	0.501	1.039
PF, mg/ml	3.84	3.86	0.923	0.038
OMD,%	45.92	46.42	0.472	0.278
NDFD,%	34.300	35.00	0.250	0.331
MMP, mg	62.50	63.15	0.747	1.070
EMMP, %	42.23	42.31	0.836	0.585
ME,MJ/kg DM	5.088	5.10	0.970	0.098
DMD,%	53.22	53.90	0.186	0.239
MCP,%	1.48	1.49	0.664	0.0139
SCFA, mmole	0.4508	0.4572	0.662	00.004
NH_3-N, mg/dl	32.199	30.18	0.055	0.566

Effect of inclusion of *Lactobacillus plantarum* on chemical composition and *in vitro* degradability

Lactobacillus plantarum was used as the biological inoculant to enhance digestibility and nutritive value of paddy straw. Two treatments were considered in the present study: without *Lactobacillus* and with the inclusion of *Lactobacillus* (2.4×10^6 cfu/g).

There was found to be no significant difference with the exclusion or inclusion of *Lactobacillus* culture in DM, CP, EE, Ash, OM, ADL, Hemicellulose, Cellulose, Silica, NDICP and ADICP. The only significant variation was found in NDF, ADF and Silica. With the inclusion of *Lactobacillus*, NDF and ADF decreased significantly (69.57 and 47.73 respectively) while without the addition of *Lactobacillus* inoculant, NDF and ADF was found to be the highest (71.16 and 48.83 respectively) ($p<0.05$). Silica was highest in the treatment without the addition of *Lactobacillus* (8.84) while with the addition of *Lactobacillus*, silica declined (8.11) and hence, showed significant variation ($p<0.05$). No significant variation was found in any of the *in vitro* utilization parameters. NGP, ml was found to be numerically higher with the inclusion (38.97 ml) than the exclusion (38.43 ml) of the culture. Similarly, OMD, %, NDFD,%, ME, MJ/kg DM, MCP, mg and NH_3-N, mg/dl without and with culture was 147.06 % and 148.89 %, 34.30% and 35%, 5.08 MJ/kg DM and

Table 7: Effect of different enzymes on chemical composition of paddy straw silage

Parameters	No Enzyme	Xylanase	Cocktail	P value	SEM
DM	60.23	60.66	59.92	0.679	0.488
CP	8.71	8.83	9.13	0.398	0.139
EE	1.43	1.44	1.43	0.960	0.023
Ash	14.83	14.48	14.40	0.531	0.157
OM	85.16	85.51	85.60	0.513	0.154
NDF	70.23	70.17	70.70	0.914	0.335
ADF	48.68	48.68	47.48	0.100	0.262
ADL	6.54	6.47	6.18	0.347	0.102
Hemicellulose	21.55	21.48	23.00	0.076	0.309
Cellulose	34.04	33.90	32.89	0.312	0.353
Silica	8.57	8.59	8.27	0.627	0.124
NDICP	4.89	4.88	4.86	0.860	0.0336
ADICP	4.62	4.63	4.63	0.994	0.0312

Table 8: Effect of different enzymes on *in vitro* utilization of paddy straw silage

	Treatments				
	No Enzyme	Xylanase	Cocktail	p -value	SEM
NGP, ml	38.52	38.70	38.87	0.748	0.368
NGP, ml/g DM	102.74	103.67	103.92	0.748	0.983
TDS, mg	319.35	320.69	320.83	0.579	0.578
OMD, mg	146.85	148.53	148.55	0.805	1.039
PF, mg/ml	3.80	3.86	3.89	0.566	0.038
OMD,%	45.95	46.27	46.29	0.894	0.278
NDFD,%	34.27	34.45	35.25	0.551	0.331
MMP, mg	61.32	63.36	63.78	0.598	1.07
EMMP, %	41.47	42.46	42.87	0.515	0.585
ME,MJ/kgDM	5.05	5.10	5.11	0.904	0.098
DMD,%	53.62	53.32	53.73	0.823	0.239
MCP,%	1.48	1.48	1.49	0.990	0.139
SCFA, mmole	0.4560	0.4541	0.4519	0.748	0.004
NH_3-N, mg/dl	31.60	30.98	30.97	0.818	0.566

5.10 MJ/kg DM, 1.48 mg and 1.49 mg and 30.18 mg/dl and 32.19 mg/dl respectively. Similar results were seen by Marbun *et al*. (2020) who recorded that the *Lactobacillus plantarum* treatment increased DM and CP, reduced NDF and ADF contents compared to control and also produced more lactic acid compared to the other LAB-treated paddy straw silages.

Effect of inclusion of enzymes on chemical composition and *in vitro* degradability

With the intent of exploiting the lignocellulosic breakdown by fiber degrading enzymes, Xylanase and Cocktail (mixture of cellulase, hemicellulase etc.) were used as additives @50 g/100 kg paddy straw resulting in three treatments: Without enzyme addition, addition of xylanase and addition of cocktail.

No significant variation was observed in three different treatments of enzymes in DM, CP, EE, Ash, OM, NDF, ADF, ADL, Cellulose, Silica, NDICP and ADICP. CP and OM were numerically higher with addition of cocktail. ADF, ADL, Cellulose and Silica showed a numerical decrease with the inclusion of cocktail. No significant variation was found in any of the *in vitro* utilization parameters. NGP, ml was found to be 38.52 ml, 38.70 ml and 38.87 ml with the treatments of no addition of enzyme, xylanase and cocktail addition respectively. The PF value (mg/dl) was recorded as 3.80, 3.86 and 3.89 in the subsequent different treatments. MMP, mg was found to be 61.32, 63.36 and 63.78 mg in the paddy straw silage with no addition of enzyme, xylanase and cocktail respectively. The results are consistent with the study conducted by Zhao *et al*. (2019) wherein at inclusion of enzyme @0.6%, the highest levels of degradation were found ($p<0.05$)

CONCLUSION

The DM, ADL, NDICP and ADICP were found to be significantly higher ($p<0.05$) and NDF, hemicellulose and cellulose showed a significant decline ($p<0.05$) with the inclusion of urea @1% and molasses@6%. *Lactobacillus* inclusion caused a significant decline in NDF, ADF and silica ($p<0.05$). Evaluation of *in vitro* utilization showed significantly higher ($p<0.05$) NGP, ml, OMD, mg, OMD, %, DMD, % , MCP, % and ME, MJ/kg DM with urea@1%, molasses@6% and with the inclusion of *Lactobacillus plantarum* and cocktail enzyme.

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