Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com
J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(6): 2626-2630

Received: 06-09-2017
Accepted: 07-10-2017

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Effect of feeding urea molasses treated rice straw along with fibrolytic enzymes on the performance of Corriedale Sheep

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Abstract
A growth trial of 60 days (March-April) was conducted on 18 male Corriedale sheep at Sheep Research Station, Shuhama, Srinagar (SKUAST-Kashmir), to study the effect of feeding of paddy straw based complete feed without (T0) or with urea molasses (T1) and fibrolytic enzyme mixture (T2) supplementation. Improvement in crude protein and reduction in ADF and NDF content of rice straw by urea molasses and fibrolytic enzyme treatment was observed. Average body weight and average daily gain of animals fed urea-molasses and enzyme treated paddy straw was significantly (P<0.05) higher than control with reduction in cost of feed per animal. Digestibility of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose digestibility were significantly (P<0.05) higher in animals of T_3 group followed by T_2 and T_1 groups. Haemato-biochemical parameters, Hb (g%) and total protein were found significantly (P<0.05) higher in animals fed enzyme and urea-molasses treated paddy straw (T_3) group than animals of T_1 and T_2 groups. The mean values of TVFA concentrations, total nitrogen and NH_3-N recorded at different hours post feeding were found significantly (P<0.05) higher in treatment groups than that of control; with non-significant difference in the rumen pH, TCA ppt. N (mg/dl) and NPN values of the treatment groups.

Keywords: Fibrolytic enzymes, rice straw, urea molasses, Corriedale Sheep

1. Introduction

The major constraint for the development of small ruminant production in Kashmir is shortage of feed/fodder particularly in the winter season. The conventional feed resources like pastures are no longer available due to competition of land usage and due to seasonal availability. Ruminant livestock raised in this region, therefore tend to reflect the cyclical variation in quantity and quality of these available forages. Rice straw being the principal crop residues is available all the year around in large quantities in the region and can be used as the main bulky feed for ruminant animals. Rice straw is a main agricultural byproduct which farmers usually stored for use as ruminant feed in tropical area. Rice straws contain low nitrogen, vitamins and minerals, which hinder the availability of cellulose to be degraded by rumen microbes and eventually limit the necessary nutrient uptake for a satisfactory performance of animals, especially ruminants. The cellulose and hemi-cellulose content of rice straw range between 25 - 45% and 18 - 30%, respectively, while the lignin content is between 10-15% [1-6]. Although, ruminants are endowed with the ability to convert low quality feed into high quality protein and utilize feeds from land not suitable for cultivation of crops, however, the utilization of these low quality crop residues is hampered by its low protein content, fibre, digestibility, vitamin and minerals [7].

Despite the limitation due to low nitrogen and high concentration of fibrous materials, which limit the degradation process [6], many studies have been conducted with efforts dedicated towards attaining most of the potential nutritive value of this abundant agricultural byproduct. Various treatment methods have been used to improve nutritive value of rice straw including physical, biological and chemical treatment [8]. Urea treatment is a conventional technique for improving the quality of rice straw in terms of increasing the nitrogen content [9-11] digestibility [12-17] and appear to be the most practical for use on farmers level, as the chemicals are relatively cheap and the procedures to use them are relatively simple. However, the low voluntary feed intake when using urea treated rice straw as a sole feed, resulted in the low nutrient intake (energy and crude protein) because of low dry matter intake in sheep leading to low growth rates and poor reproductive performance [18]. Hence, to utilize these treated crop residues in appreciable quantum, use of molasses to improve palatability and energy content along with biological agents (feed additives) to maximize advantage from given feeds in animal system as rice straw being high in structural carbohydrate content (lignin-cellulose and hemicelluloses). Using a mixture of urea and molasses has the advantage of reducing strong odor of free ammonium or ammonium carbonate and treatment time. Molasses also increases palatability of diet and acts as readily available source of energy for ruminal microflora for better utilization of ammonical nitrogen for protein synthesis. The use of combinations of fibrolytic enzyme with these pre-treatments is expected to have a synergistic effect on the nutritive improvement of rice straw. So the present study was undertaken to assess the effect of urea molasses treatment of rice straw along with fibrolytic enzyme mixture on feed intake, growth rate, digestibility, rumen parameters and economics in sheep.

2. Materials and Methods

A growth trial of 60 days was conducted on 18 male Corriedale sheep (1 year old, 23.00-25.35 kg) divided in three groups of six animals each, to study the effect of feeding of paddy straw based complete feed without (T0) or with urea molasses (T1) and fibrolytic enzyme mixture (T2) supplementation. A complete feed was prepared containing paddy straw 60 parts and concentrate mixture 40 parts on dry matter basis (ICAR 2013). Urea molasses treatment of paddy straw was done using 2% urea and 5% molasses in 40 liters of water (9), whereas, exogenous fibrolytic enzyme mix (cellulase, amylase, protease, pectinase, β-gluconase, lipase, phytase, mannase and xylanase) was incorporated in complete feed @ 9 g/kg DM, as per the *in vitro* studies carried to arrive at optimum level of incorporation of probiotic mix (1). The representative samples of feeds offered and residue collected were sampled, and analyzed daily for dry matter content to assess average DM consumption during the experimental period. At the end of experiment seven day metabolism trial was conducted to assess the digestibility of nutrients and balance of nitrogen and calcium and phosphorus by Talpatra *et al.* [19]. Samples of feed offered and their residues left, faeces and urine were analysed for proximate and cell wall constituents as per AOAC (2005) and Van Soest *et al.* [20] method. Economics of feeding was determined on the basis of cost of experimental feed (per 100 kg feed prepared), average total feed consumed by the animals of each group and total body weight gain by the animal in the feeding trial.

3. Statistical analysis

The data obtained from the experiment was processed and analyzed statistically using the Statistical Package for the Social Sciences, Base 14.0 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, USA).

4. Results and Discussion

Chemical composition of experimental diets

The proximate parameters were analyzed to determine the nutritive values of untreated rice straw, urea-molasses treated rice straw with and without fibrolytic enzymes (Table 1). There was improvement of crude protein and reduction in ADF and NDF content of rice straw by urea molasses and fibrolytic enzyme treatment. This improved in CP content of treated paddy straw value was similar to those values reported by Polyorach and Wanapat [21], Sheikh *et al.* [9], Wanapat *et al.* [5], Wanapat [8] and Zaman and Owen [22]. The decrease in NDF and ADF contents of the treated straw was in agreement with Samsudin *et al.* [23], Fazaeli *et al.* [24] and Singh *et al.* [25]. It is assumed that the activities of fibrolytic enzymes such as cellulase, β-glucosidase and xylanase had reduced the lignocellulose compound of the rice straw as reported by several studies previously [26-27].

Growth performance

Change in body weight gives reliable measure of performance of animals fed on different diets. The findings under the investigation (Table 2) revealed significant ($P<0.05$) difference in the mean final body weight of animals fed urea-molasses and enzyme treated paddy straw (T_1, T_2 and T_3) than control (T_1) group (Table 2). The animals of all the groups maintained their body weight and achieved a reasonable growth; with ADG significantly ($P<0.05$) higher in treated groups than control. The reduction in cost of feed per animal was found to be 2.46 and 11.69% in T_2 and T_3, respectively in comparison to T_0. These reports are in accordance with earlier reports of Jafari *et al.* [28] and Beauchemin *et al.*[29] reported better growth performance of animals fed urea treated paddy straw. Gado *et al.* [30] found Ossimi male lambs fed paddy straw ensiled orange pulp treated with exogenous enzymes highest average daily gain (ADG) than control. This trend was consistent with the results of Bassiouni *et al* [31] and Khattab *et al.* [32] who also found similar effect of fibrolytic enzymes supplementation.

DM intake and digestibility

Dry matter intake and FCR presented in (Tables 2) revealed significantly higher ($P<0.05$) dry matter intake of treatment groups than control, with values of FCR lower in treatment groups $_2$ and T_3) than control (T_1) as earlier reported by Qingxiang [33] with slightly increase dry matter intake in dairy cows fed urea treated rice straw. Enzyme supplementation is often accompanied by increased feed intake [34-36] which may partly be due to increased palatability of the diet due to sugars released by pre-ingestive fibre hydrolysis.

Regarding nutrient digestibility, dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose digestibility were significantly ($P<0.05$) higher in animals of T_3 group followed by T_2 and T_1 groups. However, digestibility of EE and CF were significantly ($P<0.05$) lower in T_1 and T_2 groups as compared to T_3 group with non-significant effect on digestibility of NFE and hemicellulose. These results are in accordance with Gomaa *et al.* [26] and Wanapat *et al.* [37]. The urea chemically break the ester bonds between hemicelluloses, cellulose and lignin and physically make structural fibers swollen, enable rumen microbes to attack the structural carbohydrates more easily hence higher degradability with higher intake[38] using fibrolytic enzymes in combination with other pre-treatments increased degradability and *in vitro* fermentation characteristics, as shown by Eun *et al.* [39] who treated with xylanase or cellulase in combination with ammonia, by Liu and Ørskov [40] who treated with cellulase from *Penicillum funiculosum* in combination with steam pre-treatment, and by Wang *et al.* [41] who treated with multi-enzymes (xylanase, β-glucanase, carboxymethylcellulase and amylase) in combination with NaOH.

All the animals subjected to various treatment groups including control were in positive nitrogen (N), Calcium (Ca) and phosphorus (P) balance. The average values of mineral balance (N, Ca and P) as g/d were found significantly higher in T_3 group followed by T_2 and T_1 group. Similar findings were also reported by Gomaa *et al*. [26] and Paul *et al*. [42] in lambs fed urea treated paddy straw. Saleem *et al.* [43], Singh and Das [44-46] and Ganai [47] observed that feeding fibrolytic enzymes to kids and lambs improved the digestibility of diet and reduced the faecal excretion of N Ca, and P fractions with numerically higher balance of these minerals.

Hemato-biochemical parameters

Regarding hemato-biochemical parameters as depicted in (Table 4), Hb (g%) and total protein were found significantly ($P<0.05$) higher in animals fed enzyme and urea-molasses treated paddy straw (T_3) group than animals of T_1 and T_2 groups as the use of alkali agents alone or along with exogenous enzymes improves utilization of nutrients in the feed through its effect on their degradation and absorption. Gomaa *et al*. [26] also reported that lambs fed urea-molasses treated paddy straw has higher plasma total protein. However, there was non-significant difference between mean values of PCV (%), blood glucose concentration (mg/dl), blood urea nitrogen (mg/dl) and serum creatinine (mg/dl) in animals of different treatment groups as earlier reported by Salem *et al.* [43], Rivero *et al.* [48] and Ganai *et al*. [49], that feeding exogenous enzymes in growing lambs had no significant effect on blood parameters, measured as an indicator for animal health.

Rumen fermentation parameters

The mean values of TVFA concentrations, total nitrogen and NH_3-N recorded at different hours post feeding (Table 4) were found significantly ($P<0.05$) higher in treatment groups than that of control; with non-significant difference in the rumen pH, TCA ppt. N (mg/dl) and NPN values of the treatment groups as treatment of paddy straw with urea and enzyme resulted in higher ruminal digestion due to increased activity of fibrolytic bacteria which is confirmed by higher DM, OM and fibre digestibility. These reports are in accordance with Gomaa *et al.* [26] who reported that lambs fed urea-molasses treated paddy straw has higher ruminal TVFA, and total nitrogen concentrations. Earlier *in-vivo* experiments conducted in calves [50, 45], goats [51] and kids [44] also reported higher TVFA concentration with no significant change in rumen pH, TCA ppt. N and NPN values to be in enzyme treated group than in untreated group.

Table 1: Feed ingredients and chemical composition of dietary treatments used in the experiment.

Item	Treatment			
	Concentrate mixture	**Un-treated Paddy straw**	**Urea molasses treated Paddy straw**	**Urea molasses + Enzyme treated paddy straw**
Ingredients				
Maize	20			
Wheat bran	20			
Deoiled rice bran	10			
Rice bran	10			
Soyabean	14			
Mustard oil cake	18			
Molasses	5			
Salt	1			
Mineral mixture	2			
Chemical composition (% of dry matter)				
DM	88.00	94.00	52.34	78.98
CP	21.50	3.50	6.40	6.48
EE	4.50	1.50	1.56	1.55
NDF	15.70	84.90	73.9	71.98
ADF	8.40	61.30	53.9	51.87
Ash	4.60	4.90	6.12	4.98
AIA	2.40	3.10	3.21	3.44
Ca	0.98	0.41	0.43	0.42
P	0.72	0.21	0.22	0.22

Note: Mineral mixture consisted of Vitamin A-7,00,000 I.U, Vitamin D_3-70, 000 I.U, Vitamin E-250mg, Nicotinamide-1000mg, Co-200mg, Cu - 2000mg, I - 325mg, Fe - 1500mg, Mg - 6000mg, Mn - 1500mg, K - 100mg, Na - 5.9mg, S - 0.72%, Zn - 15gm, Ca - 25% and P - 12.75%

Table 2: Dry matter intake, bodyweight, feed conversion and economics in different treatment groups

Particulars	Treatment groups		
	T_1 (Control)	**T_2** (urea-molasses)	**T_3** (urea-molasses+ enzyme)
Dry matter intake			
DMI (g/d) **	745.77 ±12.39 ^{a}	794.15 ±12.42 ^{b}	803.19 ±12.89 ^{b}
Body weight			
Average Body weight (kg)**	26.04 ±0.29 ^{a}	26.49 ±0.35^{ab}	27.15 ±0.37^{b}
Average daily gain (g/d)**	68.11 ±2.21 ^{a}	76.72 ±2.67 ^{b}	87.36 ±3.04 ^{c}
FCR**	11.26±0.46 ^{b}	10.71±0.48 ^{ab}	9.50±0.41 ^{a}
Reduction in feed cost/ animal (%)	-	2.46	11.69

^{abcd}Means superscripted with different letters in a row for a particular data differ significantly from each other *($P<0.05$), **($P<0.01$)

Table 3: Average nutrient digestibility and mineral balance in different treatment groups

Attribute	Treatment groups		
	T1 (Control)	T2(urea-molasses)	T3 (urea-molasses+ enzyme)
DM	54.27±0.29 [a]	62.12±0.17 [b]	67.28±0.36 [c]
CP	55.14±0.24 [a]	60.23±0.27 [b]	64.46±0.21[c]
EE	64.40±0.39 [a]	65.08±0.81[a]	68.23±0.24 [b]
CF	60.18±0.42 [a]	63.83±0.41[ab]	67.15±0.47 [b]
NFE	68.11±0.29	68.92±0.71	69.20±0.23
NDF	50.47±0.23[a]	54.24±0.25[b]	58.65±0.22[c]
ADF	60.22±0.51[a]	64.24±0.22[b]	68.93±0.58[c]
HC	62.51±0.26	63.37±0.48	63.99±0.32
Cellulose	60.17 ±043[a]	62.43 ±0.68[a]	64.68 ±0.92 [b]
N Balance			
(g/d) **	3.52±0.79[a]	4.20±0.23[b]	5.33±0.10[c]
% retention**	28.42±0.03[a]	26.90±0.05[a]	33.06±0.24[b]
% absorbed**	63.77±0.06[a]	67.25±0.09[b]	68.96±0.10[b]
Ca Balance			
(g/d) **	3.94±1.04[a]	4.54±0.54[b]	5.10±0.94[c]
% retention**	37.98±0.49[a]	39.66±1.32[ab]	43.36±1.99[c]
% absorbed**	59.21±0.87[a]	65.51±0.65[b]	67.14±0.87[b]
P Balance			
(g/d) **	2.26±0.62[b]	1.76±0.12[a]	2.34±0.76[b]
% retention**	57.38±0.55	51.03±1.21	60.32±1.08
% absorbed**	69.07±1.02	62.88±1.05	70.12±1.04

[abcd]Means superscripted with different letters in a row for a particular data differ significantly from each other *($P<0.05$), **($P<0.01$)

Table 4: Haemato-biochemical and rumen fermentation parameters of animals in different treatment groups

Period (days)	Treatment groups		
	T1	T2(urea-molasses)	T3 (urea-molasses+ enzyme)
Haemoglobin (g%)	11.43 ±0.11[a]	11.23 ±0.11[a]	11.98 ±0.07 [b]
Packed cell volume (%)	35.00±0.14	35.40 ±0.31	35.30±0.30
Blood glucose (mg/dl)	62.00±1.08	62.50±1.11	63.16±1.31
Total protein (mg/dl)	7.07±1.23[a]	7.87±1.00[a]	8.12±0.95[b]
Blood urea nitrogen (mg/dl)	4.40±1.11	4.30±1.23	4.17±1.19
Serum creatinine (mg/dl)	2.97±0.98	3.27±0.34	3.05±0.21
Rumen fermentation parameters			
pH	6.70±0.02[ab]	6.40±0.02[a]	6.64±0.02[a]
TVFA(mEq/l)	74.50±0.96 [a]	78.49±0.97 [b]	79.38±1.11[b]
Total Nitrogen(mg/l)	76.11±3.33[a]	80.17±2.33[b]	80.53±3.71[b]
NH_3-N (mg/dl)	21.63±0.47[a]	22.02±0.23[b]	23.66±0.71[b]
TCA ppt. Nitrogen (mg/dl)	49.50±1.44	50.12±0.87	50.69±1.11
NPN (mg/dl)	52.78±1.76	52.10±1.54	52.44±1.68

Means superscripted with different letters in a row (ab) for a particular data differ significantly ($P<0.05$).

5. Conclusion
Based on the results of study regarding nutrient improvement, body weight gain, feed intake, nutrient digestibility, heamato-biochemical and rumen fermentation parameters implications could be made that utilization of paddy straw could be increased with urea molasses and enzyme treatments and the maximum utilization is achieved by combination of both urea-molasses and enzyme treatment without any adverse effect on animal health.

6. Acknowledgments
The authors are thankful to Incharge, Sheep Research Station, F.V. Sc. & A.H., SKUAST-Kashmir for providing necessary facilities.

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