

Effect of Substitution of Urea With Different Type and Levels of Ruminant Manure on Nutritive Value of Rice Straw Silage

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Abstract : This study was conducted in Nutrition Lab. to investigate the effect of the type and level of substitution of urea with ruminant manure, M (sheep, cow and buffalo) on basis of nitrogen (N) content on the nutritive value of rice straw silage (RSS). Accordingly silages were nominated as, S-RSS, C-RSS and B-RSS. Urea (U) was substituted with dried manure at 6 combinations, 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 of U:M. Silage samples were prepared by treating chopped straw with pre-treated solution contained 10% low quality debris and 2% urea. Results showed that lower ($P<0.01$) DM loss (11.4%) was observed in S-RSS, and with addition of urea only (3.6%).

Samples of S-RSS and C-RSS recorded higher ($P<0.01$) Fleig points (Fp) as compared with those prepared by the addition of B-RSS, 60.42, 55.58 and 49.59 respectively. Reduction ($P<0.01$) in this parameter was noticed in samples prepared with a combination of 100:0. Aerobic stability (AS) was a reduced ($P<0.01$) in samples prepared by addition of S-RSS by 15 and 13 hours in comparison with samples of C-RSS and B-RSS respectively. Samples prepared with combination of 100:0 were prior ($P<0.01$) as compared with other samples.

Results also showed an increase ($P<0.01$) in in vitro digestibility of organic matter (IVOMD) in samples of S-RSS in comparison with samples prepared by addition of C-RSS and B-RSS, 49.99, 44.59 and 42.77% respectively. Samples prepared with combination of 100:0 recorded lower ($P<0.05$) in vitro digestibility of dry matter (IVDMD) as compared with combinations of 70:30 and 60:40 of U: M, 40.52, 45.36 and 45.94% respectively.

Key words: Rice straw, silage, quality, fermentation, digestibility.

I. INTRODUCTION

Roughages are the main source of ruminant diets. It was expected that these animals will maximize their dependence on roughages due to expansion of human activities and high competition on concentrate diets. Straws are the most important roughages because huge amounts are available as a byproducts of cereal planting. Low nutritive value due to its high structural carbohydrates (SC) and low protein (CP) contents reduces benefits of these materials to ruminants (1). Rice straw (RS) is an important source of diet for ruminants in many rice producing lands.

Breaking down complex structure of carbohydrates and increasing nitrogen content should be involved in any effort aims to improve the low quality of straws. Many attempts were performed to ensure that goal including the use of chemical treatments particularly with basics achieved good results regarding chemical composition due to exposure of mass to the chemical effect. However, low palatability of treated straws was still unsolved problem and grew up in many cases in which health signs may appear (2).

Ensiling if well done can be a successful alternative (3). Manures are considered as important sources of nutrients such as nitrogen and with suitable methods of handling can be introduced in ruminant diets without negative effects on the overall palatability and animal health. Moreover, using animal manure in feeding not only preserves nutrients but it participates in minimizing pollution caused by accumulation of these materials (4). Therefore, this study aimed to investigate the possibility of improving low quality of rice straw by ensiling to make use of accepted odor of good quality silage as well as to improve the chemical composition by the addition of urea and manure.

II. Materials and Methods

1. Preparation of silage samples

This study was conducted in the Nutrition laboratory from December 2015 to November 2016 to prepare samples rice straw silage (RSS) by addition of urea and ruminant manures, sheep, cows and buffalo. Accordingly silages were nominated as, S-RSS, C-RSS and B-RSS. Straw was chopped into 1-2 cm and treated with treatment solution. This solution was prepared by addition of 10% of low quality debris as a source of water soluble carbohydrates (WSC) and commercial urea (U) as a source of nitrogen (N) at rate of 2%. Manures (M) were dried at field to reduce moisture and at 105 C° for 24 hours using air draft oven. Dried manures were added to substitute different ratios of urea on basis of N content. Accordingly 6 combinations were formed of U:M, 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50. Treatment solution was diluted with estimated quantity of water to adjust dry matter (DM) content to about 40%. Each manure was mixed with treatment solution and sprayed on straw. Components of each silage treatments were packed in double plastic bags which were well compressed to exclude air and were tightly closed and stored underground for 60 days.

2. Chemical analysis

Rice straw, manures, additives and silage samples were analyzed for proximate analysis (5). Fiber fractions were analyzed according to Goering and Van Soest (6). Chemical composition of straw and manures is shown in table 1.

3. Determination of Quality characteristics

Dry matter (DM) loss was estimated as a percentage difference in total content of DM of RSS before and after ensiling (7). Fleig points (Fp) were calculated by equation reported by Kilic (8); $Fp = 220 + (2 \times DM\% - 15) - 40 \times pH$, where, Fp values of 80-100, 60-80, 40-60, 25-40 and <25 were classified as very good, good, moderate, satisfying and worthless qualities. Aerobic stability (AS) as a time required to raise silage temperature by 2C° above ambient temperature, was determined by method of Levital, et. al., (9), where, 120 g of samples were thoroughly shaken to ensure air exposure and then packed loosely in 500 ml plastic container. Samples were covered with double-layered cheesecloth to prevent drying. Four small holes were made on top of each container to permit air exchange.

Thermometer was inserted inside silage mass. An additional container filled with water to measure ambient temperature. Temperature of silage was recorded every 30 min. Buffering capacity (BC) was determined as described by Playne and McDonald (10). Twenty g of fresh material was macerated with 250 ml of distilled water. The pH of the macerate was recorded. The macerate was titrated first to pH 3 with 0.1 N HCl in order to release bicarbonate as carbon dioxide (R₁), and then was titrated to pH 6 with 0.1 N NaOH (R₂). Buffering capacity was expressed as Meq. of alkali required to change the pH from 4 to 6 per 100 g of DM, after correction for the titration value of a 250 ml water blank: $BC (meq. NaOH/100g DM) = 390 / (R_2 - R_1) \times DM\%$ of sample. In vitro DM and OM digestibilities (IVDMD, IVOMD) of RSS samples was determined as described by Tilley and Terry (11).

Statistical analysis: Data obtained were analyzed as a factorial experiment in completely randomized design by analysis of variance using statistical analysis system, SAS (12).

Table 1- Chemical composition of rice straw, silages and additives (%)

Nutrients (%)	Rice straw	Urea	Debris	Manures		
				Sheep	Cow	Buffalo
DM	91.25	-	68.75	97.44	96.55	95.81
Ash	19.75	-	2.57	12.74	21.01	24.14
CP	3.15	* 287.5	2.20	7.81	4.35	4.14
EE	4.53	-	-	3.26	3.37	3.46
NDF	79.44	-	-	74.78	79.95	78.50
ADF	51.02	-	-	41.61	40.33	39.87
ADL	8.17	-	-	10.80	9.27	9.04
Cellulose	42.85	-	-	30.81	31.06	30.83
Hemicellulose	28.42	-	-	33.17	39.62	38.63
IVDMD	39.32	-	-	48.64	49.62	46.11
IVOMD	41.20	-	-	55.61	49.91	48.14

*46 × 6.25; DM=dry matter; CP=crude protein; EE=ether extract; NDF=Neutral detergent fiber; ADF=Acid detergent fiber; ADL=acid detergent lignin; IVDMD=in vitro dry matter digestibility; IVOMD=in vitro organic matter digestibility

III. Results and discussion

Table 2 shows the effect of type of manures (M) and different combinations of urea: manure (U:M) on characteristics of the quality and nutritive value of rice straw silage (RSS). Statistical analysis revealed that DM loss, Fp, AS and IVOMD were significantly ($P<0.01$) affected by type of M, lower DM loss was estimated in S-RSS (11.40%) as compared with C-RSS (15.47%) and B-RSS (15.01%). DM loss may be occurred due to nature and rate of degradation of nutrients in RS during ensiling as affected by type of M. This was evidenced by a decline in the concentration of ammonia nitrogen ($\text{NH}_3\text{-N}$) during ensiling (as it represents the end product of degradation of CP) in S-RSS as compared with C-RSS and B-RSS (13).

Jarrige, et. al (14) attributed DM loss to the dissociation of non-fatty organic compounds such as WSC and CP. Effluents were considered as one of main source of loss in silage nutrients. Savoie, et. al., (15) reported that effluents may account for 16% of DM loss. Alves, et. al., (16) confirmed the correlation between DM loss and rate of fermentation of ensiled materials after the removal of oxygen.

Failure in maintaining anaerobic conditions during ensiling leads to secondary fermentation, in which organic matter is aerobically oxidized with large quantities of effluent accumulated beneath silage mass (17). This case is undesirable due to producing unpalatable low quality silage with high DM loss (18).

Results revealed that there was a significant ($P<0.01$) decrease in DM loss when samples of RS silage prepared with a U:M combination of 100:0 as compared with other combinations, 90:10, 80:20, 70:30, 60:40, 50:50, the differences were, 9.8, 7.52, 15.84, 13.58 and 15.44% respectively. This may be attributed to slowdown or embedding of silage fermentation as affected by ammonia produced from dissociation of urea (7).

Unlike a decline in DM loss with increasing level of urea in combination of U:M, Al-Sultani (19) observed that DM loss was increased with high level of urea treatment. The inconsistency between both studies may be due to the type of ensiled materials, common reed and rice straw. In addition to the role of manures incorporated in the current study to substitute gradually part of urea.

Results showed that there was a significant ($P<0.05$) decrease in buffering capacity (BC) by 5.14 Meq NaOH/100 g DM in S-RSS as compared with C-RSS and B-RSS. Since materials with higher BC are more resistant to reduction in pH and organic acids are responsible for BC of grass and silage (10). Abid (13) reported that lower BC of S-RSS can be explained by lower pH values and $\text{NH}_3\text{-N}$ concentration as a percentage of total N (TN) (5.04 and 0.642) recorded in samples of S-RSS as compared with 5.17 and 0.679 in C-RSS and 5.31 and 0.682 % of TN in B-RSS.

Results also showed that there was a significant ($P<0.01$) increase in BC in RSS ensiled with U: M combination of 100:0 as compared with the other combinations which were not significantly differed. Higher BC in that case may be due role of ammonia produced from dissociation of urea during ensiling in extension fermentation time. Buffering effect of ammonia equalized any increase in production of total acids. Therefore, addition of WSC such as molasses is highly recommended to improve silage quality (20). Ghazali, et. al., (21) confirmed that addition of urea at ensiling increase BC.

Aerobic capacity (AC) affects the characteristics of quality and nutritive value, hence it is considered one of important parameters of silage fermentations. Danner, et. al., (22) demonstrated that AS affects ability of silage to resist against aerobic spoilage and oxidation of end products of anaerobic fermentations caused by the attack of aerobic microbes soon after the open of silo. Results of current

study revealed that AC of S-RSS was significantly ($P<0.01$) decreased by 15 and 16 hours in comparison with that of C-RSS and B-RSS respectively.

Regarding the effect of U:M combinations on AS, results showed that RSS samples prepared with a combination of 100:0 were more aerobically stabled ($P<0.01$) by 32.67, 52.67, 71.34, 100.67 and 116.67 hours over those prepared with other combinations, 90:10, 80:20, 70:30, 60:40 and 50:50 respectively. As shown, urea may play a conclusive role in promoting AS of RSS, therefore, AS values were adopted ascending trend with increasing urea level in these combinations of urea: manure. Oud Elferink, et.al., (23) found that urea and ammonia participated in improving AS of silage. This effect was attributed to its antifungal effect (24). Consequently, the decline in AS in RSS samples with increasing level of manures at the expense of urea can be explained on basis of the negative effect of fermentation acids, particularly homofermentation of lactic acid (25). Because lactic acid per se is non-effective antifungal agent (26). This acid can be metabolized by yeasts as soon as silage was exposed to air (27).

In vitro digestibility of dry matter (IVDMD) was not affected by the type of manures in the current study, IVDMD values were, 45.20, 43.00 and 42.66% for S-RSS, C-RSS and B-RSS respectively. Slight increase in S-RSS may be due to its higher content of protein as compared with C-RSS and B-RSS (7.81 vs. 4.35 and 4.14%, respectively, table 1). Sundstl and Owen (28) mentioned to role of N in enhancing ensiled materials. However, IVDMD of all RSS samples were improved as compared with IVDMD of RS before ensiling (39.32%, table 1) regardless to the type of manures. This may be derived from providing silage microbes with additional nitrogen.

Results also pointed out that lower ($P<0.05$) IVDMD was recorded in RSS samples prepared with U: M combination of 100:0 as compared with those prepared with combinations of 70:30 and 60:40, IVDMD values were, 40.52, 45.36 and 45.94% respectively. Improvement of IVDMD in these combinations in comparison with 100: 0 of U: M may be due to increase the activity of silage microbes to make use of additional carbohydrates available through manures (29), together with slower degradable nitrogen than urea (30). Similar conclusion was supposed by Saeed (4) in his study on the effect of addition of dried poultry manure on nutritive value of wheat straw silage. The worker referred to close correlation between rate of utilization of nitrogen and carbohydrate in silage by silage microbes.

In vitro organic matter digestibility showed similar trend as IVDMD. Higher ($P<0.01$) IVOMD was recorded in S-RSS as compared with C-RSS and B-RSS, IVOMD values were, 49.99, 44.59 and 42.77% respectively. Improvement of IVDMD in S-RSS can be explained by differences in CP content among the type of manures (table 1). Preparing RSS samples with different combinations responded in different improvements in IVOMD as compared with IVOMD of RS before ensiling as a result of stimulation of bacterial growth due to increase N supply (31). However, higher ($P<0.01$) values were recorded in RSS prepared with U: M combinations of 50:50 and 60:40 (46.82 and 46.78%, respectively) in comparison with those prepared with a combination of 70:30 (46.20%) which exceeded those prepared with combinations of 80:20 and 90:10 (45.61 and 45.75% respectively).

This result refers clearly to the role of the increased level of manure in U: M combinations in the improvement of IVOMD, which is associated with degradation rate of N in manures in comparison with fast degradation rate of urea, where, lower ($P<0.01$) IVOMD was recorded in RSS prepared with U: M combination of 100:0 (43.56%). Effect of interaction between type of manure and U: M combinations on nutritive value are shown in table 3. Statistical analysis of data revealed that DM loss was significantly ($P<0.01$) affected in favor of RSS prepared without addition of manure (100:0 of U: M combination). Lower ($P<0.01$) DM loss (3.6%) was estimated in that treatment as compared with others especially those prepared with SM at U:M of 70:30 and 50:50 (18.29 and 19.10%, respectively) and CM at U: M combinations of 70:30, 60:40 and 50:50 (19.36, 25.13 and 22.54% respectively) and BM at combinations of 80:20, 70:30 and 60:40 (19.34, 20.66 and 19.58% respectively). No significant difference were observed among treatments prepared with S-RSS at combination of 80:20 and 60:40 and C-RSS at combination of 80:20. Mode of changes in DM loss as affected by interaction effect of M and U: M may be derived from slowdown and embedding of fermentations in silos caused by ammonia produced from dissociation of urea (17).

Table.2:Effect of type of manures and combinations of urea: manure on quality characteristics and nutritive value of rice straw silages (as appeared ± SE)

Items	M			Combinations of U: M (%)						P	
	SM	CM	BM	100:0	90:10	80:20	70:30	60:40	50:50	M	U:M
DM loss, %	11.40 ^b ±1.32	15.47 ^a ± 1.49	15.01 ^a ± 1.26	3.60 ^c ± 0.11	13.40 ^b ± 1.36	11.12 ^b ±1.89	19.44 ^a ± 0.58	17.18 ^a ± 2.20	19.04 ^a ± 0.94	**	**
Fp, points (pts.)	60.42 ^a ± 2.67	55.85 ^a ± 1.96	49.59 ^b ± 2.37	39.79 ^b ± 2.90	59.12 ^a ±3.86	56.60 ^a ± 3.75	56.75 ^a ± 1.64	62.00 ^a ± 3.30	57.46 ^a ± 2.07	**	**
BC, Meq NaOH/100 g DM	65.01 ^b ± 2.30	70.15 ^a ± 2.00	70.15 ^a ± 1.86	80.11 ^a ± 3.36	73.53 ^{ab} ± 2.20	70.57 ^{bc} ± 2.50	65.80 ^{cd} ± 2.05	59.91 ^d ± 1.69	60.70 ^d ± 2.04	*	**
AS, hours (hrs.)	363.33 ^b ± 7.40	378.33 ^a ± 7.67	379.33 ^a ± 7.66	436 ^a ± 3.57	403.33 ^b ± 4.15	383.33 ^c ± 4.32	364.66 ^d ± 3.76	335.33 ^e ± 2.04	319.33 ^f ± 1.30	**	**
IVDMD, %	45.20 ± 0.83	43.00 ± 1.06	42.66 ± 1.04	40.52 ^c ± 0.68	44.43 ^{abc} ± 1.46	41.42 ^{bc} ± 1.22	45.36 ^{ab} ± 1.76	45.94 ^a ± 1.39	44.06 ^{abc} ± 1.36	NS	*
IVOMD, %	49.99 ^a ± 0.65	44.59 ^b ± 0.20	42.77 ^c ± 0.13	43.56 ^d ± 0.09	45.75 ^c ± 0.90	45.61 ^c ± 0.88	46.20 ^b ± 1.10	46.78 ^a ± 1.02	46.82 ^a ± 1.09	**	**

Means having different letters at the same row are significantly different at * (P<0.05) ** (P<0.01)

DM loss/ dry matter loss

Fp, pts. Fleig points

AS, h./ Aerobic stability, hours

BC/ Buffering capacity

IVDMD/ In vitro dry matter digestibility; IVOMD/ In vitro dry matter digestibility

SM/ Sheep manure; CM/ Cows manure/ BM/ Buffalo manure; U: M/ Urea: manure

Fleig point was also significantly (P<0.01) affected by the above mentioned interaction. Higher Fp (76.23 pts.) was estimated in S-RSS prepared with a combination of 60:40 as compared with other treatments. No significant differences were observed among that superior treatment and S-RSS prepared with combinations of 80:20 (70.01) and 90:10 (68.73 pts.). Lower Fp was recorded in RSS prepared without addition of manure (39.79 pts.)

The above result supports the idea that ammonia (urea) embedded silage fermentation, and it was clearly reflected on DM loss as previously discussed. This agrees with the observations of Saeed (32, 33) in wheat straw silages prepared with addition of urea.

Since the estimation of Fp of silage depends on values of pH and level of DM (8), Values of Fp were varied according to variance existed in pH and DM contents of RSS.

Aerobic stability (AS) was also significantly (P<0.01) affected by interaction between type of manure and U: M combinations. Samples of RSS prepared without addition of manure (U: M combination of 100:0) stood for 436 hours before the onset of aerobic deterioration. Aerobic stability of this treatment exceeded that of other samples by 20 h. in B-RSS prepared with a U: M combination of 90:10 to 118 h. in B-RSS prepared with a U: M combination of 50:50. The superiority of AS in RSS samples prepared without addition of manure may ascribed to role of ammonia produced from dissociation of urea during ensiling in embedding growth of fungi and yeasts when ensiled materials were exposed to air (24). Hence, AS in these samples was improved as it received higher level of urea (100%), therefore, AS was decreased as the level of manure was increased in other combinations regardless to the type of manure as a result of a decline in the level of urea (ammonia).

In vitro digestibility of organic matter (IVOMD) was significantly (P<0.01) affected by the interaction between the type of manure and U: M combinations. Higher values were observed in S-RSS prepared with U: M combinations of 50:50 (52.22%), 60:40 (51.78%) and 70:30 (51.84%), whereas lower value were recorded in B-RSS prepared with combinations of 50:50, 60:40 and 70:30, values for these combinations were, 42.40, 42.50 and 42.31% respectively. This

improvements can be explained on basis of variance in CP content of manures (table 1). In addition to the better effect of SM on silage fermentation shown by Abid (13). Lower pH and NH₃-N concentration were observed in that study.

Table 3- Effect of interaction between type of manure and U: M combinations on characteristics of quality and nutritive value of reed silage (as appeared ± SE)

Item	100% Urea 100:0	SM					CM					BM					P
		U: M combinations					U: M combinations					U: M combinations					
		90:10	80:20	70:30	60:40	50:50	90:10	80:20	70:30	60:40	50:50	90:10	80:20	70:30	60:40	50:50	
DM loss	3.60 ^g ±0.21	14.75 ^{cde} 3.01±	5.87 ^g 2.04±	18.29 ^{bcd} 1.06±	6.82 ^g 1.10±	19.10 ^{bc} 1.57±	14.06 ^{de} 1.63±	8.16 ^{fg} 0.65±	19.36 ^{bc} 0.48±	25.13 ^a 2.30±	22.54 ^a 2.51±	11.40 ^e 2.51±	19.34 ^b 2.69±	20.66 ^a 1.23±	19.58 ^b 0.52±	15.48 ^c 0.70±	**
Fp, pts.	39.79 ^d ±5.43	68.73 ^{ab} 2.61±	70.01 ^{ab} 1.34±	56.43 ^{bc} 4.12±	76.23 ^a 0.98±	51.32 ^{cd} 4.65±	61.38 ^{bc} 5.19±	51.19 ^{cd} 2.16±	60.66 ^{bc} 1.06±	60.45 ^{bc} 1.75±	61.67 ^b 2.36±	47.27 ^c 7.99±	48.59 ^c 8.56±	53.18 ^c 1.80±	49.32 ^c 4.41±	59.39 ^b 1.71±	**
BC, Meq NaOH/100 g DM	80.11 ^a ±6.30	71.93 ^{ab} 3.55±	65.91 ^{bc} 5.20±	61.14 ^{bc} 4.00±	55.25 ^c 2.59±	55.77 ^c 3.17±	74.33 ^{ab} 4.49±	72.91 ^{ab} 4.55±	68.14 ^{abc} 3.44±	62.25 ^{bc} 2.01±	63.17 ^b 4.39±	74.33 ^a 4.17±	72.91 ^a 3.16±	68.14 ^a 2.86±	62.25 ^b 3.33±	63.17 ^b 2.32±	*
AS, h.	436 ^a ± 6.68	384 ^{cd} 4.33±	364 ^e 5.62±	348 ^f 3.89±	328 ^{gh} 1.78±	320 ^h 2.36±	410 ^b 3.70±	394 ^c 3.46±	374 ^{de} 4.67±	336 ^{fg} 3.17±	320 ^h 3.14±	416 ^b 2.12±	392 ^c 3.44±	372 ^{de} 2.58±	342 ^f 2.42±	318 ^h 1.30±	**
IVDMD, %	41.41 ^{bc} ±0.60	49.11 ^{ab} 1.77±	41.76 ^{bc} 1.03±	47.08 ^{abc} 1.88±	50.04 ^a 1.34±	41.79 ^{bc} 0.79±	43.05 ^{ab} 2.97±	40.91 ^c 3.49±	43.55 ^{abc} 2.29±	43.11 ^a 3.39±	47.31 ^a 1.34±	41.13 ^c 1.34±	41.59 ^b 1.53±	45.46 ^a 4.73±	44.66 ^a 0.63±	43.08 ^a 3.61±	*
IVOIM, %	43.56 ^{ef} ±0.17	50.39 ^b 0.24±	50.17 ^b 0.44±	51.84 ^a 0.35±	51.78 ^a 0.15±	52.22 ^a 0.05±	43.73 ^{de} 0.16±	43.90 ^{de} 0.13±	44.45 ^d 0.39±	46.06 ^c 0.09±	45.85 ^c 0.17±	43.12 ^e 0.57±	42.76 ^f 0.09±	42.31 ^h 0.14±	42.50 ^g 0.09±	42.40 ^g 0.28±	**

Means having different letters at the same row are significantly different at * (P<0.05) ** (P<0.01)

DM loss/ dry matter loss

Fp, pts. Fleig points

AS, h./ Aerobic stability, hours

BC/ Buffering capacity

IVDMD/ In vitro dry matter digestibility; IVOMD/ In vitro organic matter digestibility

SM/ Sheep manure; CM/ Cows manure/ BM/ Buffalo manure; U: M/ Urea: manure

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