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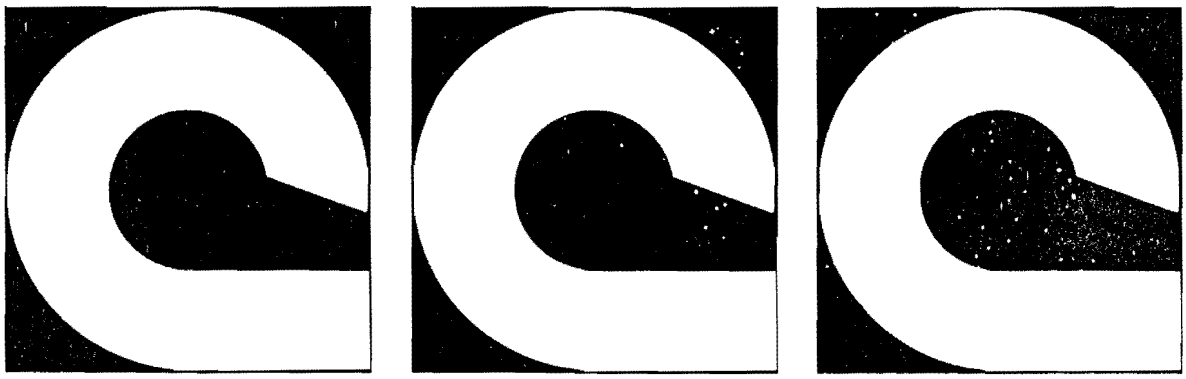

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Energy and protein utilization by goats fed Italian ryegrass silage treated with molasses, urea, cellulase or cellulase + lactic acid bacteria

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Abstract

The effects of different additives on ensiling of Italian ryegrass (IRG) silage and the resulting silages energy and nitrogen utilization and on methane (CH_4) emission by Japanese native goats were evaluated. The silages were prepared from IRG harvested at late-bloom stage. Different treatments, 13.3% molasses, 4.0% urea, 0.02% cellulase and 0.02% cellulase + 0.02% lactic acid bacteria (LAB) were mixed with IRG prior to ensiling, and compared to a control with no preservative. Diets consisted of corn and soybean meal with one of the silages. Goats were allocated to examine one of the treated diets in two metabolism trials. Each trial period lasted 21 days, with a 7-day period of adjustment, followed by a 7-day preliminary period and a 7-day period of total collection of digestion and metabolism data. Methane emission was measured in open circuit respiration chambers over three consecutive days for each goat. Urea treated IRG had a higher ($P < 0.05$) volatile ammonia nitrogen (NH_3-N/TN, 57%) compared to other silages. All treated silages had higher ($P < 0.05$) OM digestibilities except the cellulase treated silage. The cellulase + LAB treated silage diet had the lowest CH_4 kg^{-1} digestible organic matter intake (DOMI). Goats consumed similar N in all diets except the urea treated silage diet. All treated silage diets produced higher ($P < 0.01$) urinary N than that of the control diet. The CP digestibilities were similar ($P > 0.05$) in all treated silage diets except the molasses treated silage diet, where CP digestibility was the lowest. Urea treated silage diet produced a higher digestible N, but had a negative retained N due to a higher ($P < 0.001$) urinary N. Ensiling IRG with the additives led to higher digestible nutrient availability, but mixing urea resulted in increased N losses through feed, feces and urine. This study showed that IRG harvested at late-bloom stage could be ensiled without any additive, but mixing molasses, cellulase and cellulase + LAB prior to ensiling increased the proportion of ME to DE and could reduce the CH_4 emission rate per unit of DOM and retained energy (RE). Mixing urea increased the digestible OM and N intake, but increased the N excretion. Cellulase addition resulted in a decreased CH_4 production rate. However, IRG treated with molasses could increase retained energy and N besides reducing CH_4 and volatile N.

Keywords: Goat; Italian ryegrass silage; Preservatives; Methane; Energy; Nitrogen utilization

1. Introduction

Ensiling is one of the best ways to preserve green forage, but ensiling of tropical grasses is difficult primarily due to deficiency of water-soluble carbohy-

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drates (WSC) (McDonald et al., 1991). To obtain the necessary fermentable sugars for lactic fermentation in crops low in WSC, cell wall degrading enzymes (cellulase, hemicellulase and pectinase) have been suggested (Weinberg et al., 1993). Another option is to mix molasses (as a rich soluble sugar source, WSC 65% on DM basis) with tropical grasses to improve fermentation and digestibility (McDonald et al., 1991; Yokota et al., 1998). Adding urea has been shown to increase degradable N, improve the digestibility (Saadullah, 1984; Leng, 1993) and preservation of low-quality roughage (Chowdhury and Huque, 1996). Treating rice straw with urea, mineral and bypass protein reduced the estimated CH_4 production (Leng, 1993) and addition of molasses with urea treated straw decreased the in vitro CH_4 emission (Huque and Chowdhury, 1997). Use of cell wall degrading enzyme and inoculant (cellulase and LAB) as silage additive improved the fermentation quality and digestibility of Italian ryegrass, Rhodes grass and barley straw silage (Ridla and Uchida, 1997, 1999). Using molasses reduced cell wall composition and improved fermentability of napier grass silage (Yokota et al., 1991). Dietary manipulation is one of the means to reduce methane (Moss, 1994) and the effects of the additives and urea may affect the CH_4 and N production. As practicable in Asia and developing countries, the effects of these additives on CH_4 emission and N excretion are very important from the environmental pollution aspect. Therefore, the parameters should be determined. The reported studies (Saadullah, 1984; Yokota et al., 1991; Huque and Chowdhury, 1997; Ridla and Uchida, 1997, 1999) are mainly based on the in vitro analyses of feed or diet. Little calorimetric data concerning the effects of the enzymes and additives on CH_4 emission in animals are available. The objective of this study was to examine the effects of the silage additives on energy and N utilization and on CH_4 and N emission in goats using open circuit respiration chambers.

2. Materials and methods

2.1. Forage

Italian ryegrass (*Lolium multiflorum* Lam.) was seeded in late October 1998 at the National Institute of Livestock and Grassland Science (NILGS), Japan. The 15 kg of N, P, K was applied to each hectare along with 30 t ha^{-1} compost. The first crop of IRG was harvested at late-bloom (LB) stage on 25 May 1999 (137 g DM kg^{-1}). The grass was left for 24 h in the field to wilt and brought to the feed preparation room of NILGS for ensiling.

2.2. Silage preparation

Four treatments were applied prior to ensiling to the LB cut IRG: (1) with 13.3% cane molasses on DM basis of IRG (Molasses); (2) 4.0% commercial fertilizer urea (N 46%) on DM basis of IRG (Urea); (3) 0.02% cellulase (*Acremonium cellulolyticus*) on fresh basis of IRG (cellulase), and (4) 0.02% cellulase + 0.02% LAB (*Lactobacillus rhamnosus*) (cellulase + LAB). The control treatment was the same forage without any additive. Cellulase and LAB (Snow Brand Seed, Japan) were procured from Yokijirushi Shubyo Co. Ltd., Japan.

Initially one metal drum (200 l) was filled with chopped IRG and weighed to determine the capacity of the drum (45 kg). Each additive treatment was made into solution using tap water (60 ml kg^{-1} DM IRG). The required amount of chopped IRG was mixed with one of the preservative solutions, placed into the drum and pressed manually to remove as much air as possible. The drums were kept at room temperature of 24°C (21–29°C) for 60 days. After 60 days, 15 drums (three drums for each treatment) of silage were opened. A well-mixed fresh sample was taken from each of the drums and kept at −20°C for analyses. Silage for feeding trials was packed into polyethylene bags at the rate of 10 kg. After removing air from the bag, the bag opening was tied with string, and kept in a dark room at 4°C temperature.

2.3. Preparation of silage extract

About 14 g DM of silage (fresh weight) was placed into a 200 ml plastic bottle with 140 ml distilled water and the bottle was closed. The bottle was shaken gently and kept in the refrigerator at 4°C for 16–24 h for equilibration after which the extract was collected. The extract was filtered through 4 layers of gauze and no. 5 filter paper (Whatman) and the filtrate extract was used for measuring pH,

ammonia nitrogen, lactic acid and volatile fatty acids (VFA).

2.4. Animals

Eight castrated adult Japanese native goats (mean live BW 34 kg) were used in two periods in a randomized block design to evaluate the treated silages while four goats were used to evaluate control silage in one trial. Ration digestibilities and gas exchange were obtained from open circuit respiration chambers, consisting of the environmentally controlled test rooms using air conditioning system, respiration apparatus, metabolism apparatus and computer system. Detail methods were discussed by Iwasaki et al. (1982).

2.5. Diet and feeding

Diets consisted of one of the silages + corn and soybean meal to provide 1.1 times of TDN requirement for maintenance (Itoh et al., 1978). Of the total TDN requirement 70% was supplied by IRG silage and the remainder was supplied by corn and soybean meal in equal proportion. The corn contained 4536 kcal kg^{-1} DM gross energy (GE) and 81 g kg^{-1} DM CP while the soybean meal contained 4848 kcal kg^{-1} DM GE and 502 g kg^{-1} DM CP. The TDN value of the IRG silage as reported by JFS (1999) was used to calculate the amount needed to supply 70% of the goat's requirements. During the adjustment trial, two bags of silage were opened and fed to the goats. On the day before feeding trial started, the large polyethylene bags of silages were removed, and the amount of silages required for 14 days was thoroughly mixed and packed again into smaller polyethylene bags so as to feed one bag to one goat per day. For the trial II, the remaining drums were opened, the silages mixed by treatment and packed into small polyethylene bags as in trial I. The small bags were also kept in the dark room at 4 °C temperature until fed.

2.6. Laboratory analyses

Dry matter of silage was determined using the vacuum freeze-dried method. The OM and N were analyzed following the methods of AOAC (1984) and NDF was analyzed using the methods described by Goering and van Soest (1970). The silage and rumen liquor pH was determined using a glass pH electrode (Horiba F-12). The NH_3-N of silage extract and rumen liquor were determined by colorimetric analysis using the Technicom Auto Analyzer II, and VFA was determined using gas chromatograph (Hewlett-Packard HP 6890). Lactic acid concentration was determined using the capillary electrophoresis system from Hewlett-Packard (Soga and Ross, 1997). Rumen liquors were collected from individual goats at 0 and 3 h post-feeding using stomach tube. The pH was then measured immediately and then preserved in the freezer at −30 °C for further analyses.

2.7. Statistical analyses

The statistical significance of differences was determined by analysis of variance with effects for trial and diets. The diet differences were separated using Duncan's multiple range test (Gomez and Gomez, 1984). The standard error of mean differences (S.E.M.) and significant levels of diets are also presented in respective tables. The analyses were carried out using SAS (1994).

3. Results

3.1. Composition of feeds

No significant difference was found for trial but differences for diet were significant. Among the treatments, urea treated silage contained significantly highest NH_3-N, CP and NDF (Table 1). The GE contents were similar among the silages except for urea treated silage. Urea treated silage had the highest ($P < 0.001$) pH value (Table 1). Urea treated IRG had slightly higher acetic acid but a significantly lower lactic acid compared to other silages. A higher ($P < 0.01$) butyric acid concentration was in urea treated IRG silage. Total dry matter intake (DMI) and the DMI per metabolic body weight of goats in different groups were not significantly different, though slightly higher GE (MJ per day) intakes were observed in all the treated silage based diets compared to control (Table 2).

Table 1
Composition (g kg^{-1} DM unless otherwise stated) of Italian ryegrass silages treated with different additives[a]

Feeds	IRG silages					S.E.M.[b] and significance level
	Control	Molasses	Urea	Cellulase	Cellulase + LAB	
Dry matter	417 c	444 a	429 b	425 b	417 c	3.2***
Organic matter	879 c	883 b	881 c	887 a	880 c	1.16***
Crude protein	123 b	114 bc	213 a	107 c	121 b	4.37***
Ammonia nitrogen (NH_3-N)	4.6 b	2.7 e	19.4 a	3.4 c	2.8 d	0.05***
NH_3-N (% of total N)	23 b	15 d	57 a	20 c	15 d	0.99***
Neutral detergent fiber	635 a	608 c	632 a	618 b	613 bc	3.81*
Gross energy (kcal kg^{-1} DM)	4491	4471	4658	4521	4574	11.7 ns[c]
Silage pH	4.08 b	4.05 bc	7.30 a	4.11 b	3.82 c	0.12***
Lactic acid	14.84 d	19.25 b	6.66 e	16.76 c	22.91 a	0.42***
Acetic acid	9.67	9.09	11.64	9.15	9.18	1.05 ns
Propionic acids	0.398	0.650	0.661	0.478	0.515	0.13 ns
Butyric acid	0.462 b	0.399 b	1.23 a	0.515 b	0.613 b	0.21**
Acetate:propionate	24.4	14.89	21.04	19.51	18.22	5.21 ns

[a] Means with different letters in same row differ significantly.
[b] Standard error of mean differences.
[c] Non-significant ($P > 0.05$).
** $P < 0.01$.
*** $P < 0.001$.

Table 2
Digestibilities and digestible nutrient intake (g per day kg W$^{-0.75}$) in goats fed diets based on Italian ryegrass silages treated with different additives[a]

Item	Diets					S.E.M.[b] and significance level
	Control	Molasses	Urea	Cellulase	Cellulase + LAB	
Body weight of goats	26.6	30.9	29.4	28.2	32.4	3.46 ns
Total DMI (g per day)	518	616	580	566	619	52.5 ns
Roughage:concentrate ratio	3.78	3.93	4.00	4.12	4.05	0.12 ns
Gross energy (MJ per day)	9.82	11.66	11.36	10.79	11.86	1.09 ns
Digestibilities						
DM	59.6 ab	61.1 a	62.0 a	57.4 b	62.0 a	1.49*
OM	60.8 ab	63.9 a	63.1 a	58.9 b	63.5 a	1.56*
CP	75.8 a	72.3 b	78.9 a	75.8 a	76.0 a	1.58**
NDF	75.8 a	72.3 b	78.9 a	75.8 a	76.0 a	2.45*
Digestible nutrients intake						
Digestible DMI	26.34	28.87	28.5	26.02	28.36	1.10 ns[c]
Digestible OMI	23.86	26.92	25.91	24.25	25.93	1.11 ns
Digestible CPI	5.43	5.10	7.79	5.93	5.47	0.42 ns
Digestible NDFI	11.89	12.59	14.01	11.78	12.93	0.80 ns

[a] Means with different letters in same row differ significantly.
[b] Standard error of mean differences.
[c] Non-significant ($P > 0.05$).
* $P < 0.05$.
** $P < 0.01$.

Table 3
Rumen fermentation parameters in goats fed diets based on Italian ryegrass silages treated with different additives[a]

Item	Diets					S.E.M.[b] and significance level
	Control	Molasses	Urea	Cellulase	Cellulase + LAB	
Rumen pH	6.86	7.07	7.26	7.36	6.97	0.20 ns[c]
Ammonia N (mg l^{-1})	302 b	279 b	546 a	191 b	274 b	50.8***
Total VFA (mmol l^{-1})	81.9	105.3	91.0	69.0	108.9	14.01 ns
Acetic acid (mmol) (%)	64.3	66.4	65.0	64.5	64.6	2.65 ns
Propionic acids (mmol) (%)	22.1	21.1	21.1	21.0	20.5	1.53 ns
Butyric acids (mmol) (%)	9.0	8.7	9.1	8.7	9.0	0.91 ns
Acetate:propionate	2.91	3.14	3.08	3.07	3.15	0.322 ns

[a] Means with different letters in same row differ significantly.
[b] Standard error of mean differences.
[c] Non-significant ($P > 0.05$).
*** $P < 0.001$.

3.2. Rumen environment

The pH values (mean of 0 and 3 h samplings) of rumen liquor for goats fed on the diets ranged from 6.86 to 7.36 (Table 3). Among diets, urea treated diet had a slightly higher pH value. Ammonia nitrogen of rumen liquor of the urea diet was highest ($P < 0.001$; 546 mg l^{-1}) followed by control, molasses, and cellulase + LAB diet and least in the cellulase diet. Non-significant differences were found in total VFA concentration of rumen liquor. However, the concentrations were slightly higher when goats consumed the molasses and cellulase + LAB treated IRG diets. Propionoic acid concentrations were similar among the diets but a little higher concentration was found in the control diet resulting in a lower acetate:propionate in that diet (Table 3).

3.3. Diet apparent digestibility

Apparent digestibility of DM and OM were higher ($P < 0.05$) on the cellulase + LAB, urea and molasses treated IRG based diets, but the cellulase treated diet had the lowest (Table 3). The CP and NDF digestibilities were lowest for the molasses treated IRG based diet. However, the digestible DM, OM, CP and NDF intake values were similar for all the diets.

3.4. Energy balance

Gross energy intake (kcal $W^{-0.75}$ per day) was similar for all the diets (Table 4). The fecal energy (FE) losses were similar for the diets but urinary energy losses were significantly higher ($P < 0.001$) on the treated diets compared to those of the control diet. Total digestible energy (DE), metabolizable energy (ME) intake and heat production (HP) values were similar for all the diets. However, all the treated silage based diets had non-significantly higher retained energy (RE) values as compared with the control diet.

3.5. Methane production

Among diets, there was no significant difference for all the CH_4 production parameters. However, CH_4 kg^{-1} digestible OM intake values for all treated diets were slightly lower as compared with the control diet. All the CH_4 production parameters were slightly lower in the cellulase treated IRG based diet. Methane conversion ratio (MCR%, energy loss as CH_4 per unit of GE intake) for diets had small differences, but the MCR values in the treated diets were lower as compared with the control diet. The MCR values ranged from 5.10 to 5.54%, and the lowest value was found in the cellulase treated silage based diet (Table 4). The CH_4 per unit of retained energy was higher in the control diet compared with that of the treated diets. The lowest CH_4 per unit of RE was found in the molasses treated ensiled IRG based diet.

3.6. Energy partition

Energy digestibility (DE/GE), metabolizability (ME/GE) and the concentration of ME in the DM

Table 4
Energy balance (kcal per day $W^{-0.75}$, unless otherwise stated) of goats fed diets based on Italian ryegrass silages treated with different additives[a]

Item	Diets					S.E.M.[b] and significance level
	Control	Molasses	Urea	Cellulase	Cellulase + LAB	
Energy input and output						
Gross energy intake	200.2	213.2	215.1	210.2	209.3	5.33 ns[c]
Feces energy	78.7	80.6	83.8	85.5	76.3	4.71 ns
Digestible energy	121.6	132.6	131.3	124.6	133.1	6.98 ns
Urinary energy	5.1 d	7.97 c	12.8 a	9.3 bc	10.4 b	1.10***
Methane energy	11.12	11.6	11.31	10.7	11.2	0.59 ns
Methane (g 100 kg LW)	37.09	37.72	36.95	34.20	34.99	1.64 ns
MCR[d]	5.54	5.45	5.26	5.10	5.38	0.028 ns
Methane (g kg^{-1} digestible OMI)	35.26	33.18	33.14	32.45	32.09	1.56 ns
Metabolizable energy	105.4	113.1	107.2	104.6	111.4	6.45 ns
Heat production	97.1	91.4	93.4	88.4	93.4	3.89 ns
Retained energy	8.28	21.67	13.72	16.23	17.93	8.88 ns
Methane per unit of RE	1.54	0.59	0.21	0.14	−5.18	3.6 ns

[a] Means with different letters in same row differ significantly.
[b] Standard error of mean differences.
[c] Non-significant ($P > 0.05$).
[d] Methane conversion ratio.
*** $P < 0.001$.

(kcal kg^{-1} DM, M/D) were higher for the molasses and cellulase + LAB treat IRG based diets (Tables 5 and 6). Of the DE, the proportional loss as CH_4 was not affected by diets, while the proportional loss as urine was affected by the diets ($P < 0.001$). The ME as a proportion of DE was affected significantly ($P < 0.01$) by the diets and the control diet had a higher value of ME/DE compared to that of the treated

Table 5
Partition of digestible energy and metabolizable energy of goats fed diets based on Italian ryegrass silages treated with different preservatives[a]

Item	Diets					S.E.M.[b] and significance level
	Control	Molasses	Urea	Cellulase	Cellulase + LAB	
Digestibility (DE/GE)	0.61	0.62	0.61	0.59	0.63	0.024 ns[c]
Partition of digestible energy						
Urine	0.04 c	0.06 b	0.09 a	0.075 b	0.078 b	0.008***
Methane	0.091	0.088	0.087	0.086	0.85	0.005 ns
Metabolizable energy	0.87 a	0.85 ab	0.81 c	0.84 bc	0.84 bc	0.011**
ME kg^{-1} DM (M/D kcal)	2385	2396	2327	2269	2434	106 ns
ME/GE (q)	0.53	0.53	0.50	0.50	0.53	0.023 ns
Partition of metabolizable energy						
Heat production	0.92	0.81	0.90	0.85	0.85	0.085 ns
Retained energy	0.078	0.19	0.10	0.15	0.15	0.085 ns

[a] Means with different letters in same row differ significantly.
[b] Standard error of mean differences.
[c] Non-significant ($P > 0.05$).
** $P < 0.01$.
*** $P < 0.001$.

Table 6
Nitrogen (g per day $W^{-0.75}$, unless otherwise stated) utilization of goats fed diets based on Italian ryegrass silages treated with different preservatives[a]

Item	Diets					S.E.M[b] and significance level
	Control	Molasses	Urea	Cellulase	Cellulase + LAB	
Nitrogen intake	1.14 b	1.13 b	1.57 a	1.25 b	1.15 b	0.068*
Fecal nitrogen	0.27 c	0.31 ab	0.33 a	0.30 bc	0.27 c	0.012**
Digested	0.87 b	0.81 b	1.25 a	0.95 b	0.87 b	0.069*
Urinary N	0.44 c	0.77 b	1.51 a	0.82 b	0.89 b	0.07***
Retained N	0.42 a	0.05 b	−0.27 c	0.13 b	−0.02 b	0.09***
Retained/intake	0.37 c	0.04 b	−0.18 a	0.08 b	−0.02 b	0.069***
Retained/digested	0.49 c	0.06 b	−0.24 a	0.10 b	−0.026 b	0.092***
Nitrogen partition						
Fecal N/NI	0.24 b	0.28 a	0.21 b	0.24 b	0.24 b	0.015**
Urinary N/NI	0.09 c	0.68 b	0.97 a	0.68 b	0.78 b	0.06***
Total N loss/NI	0.62 c	0.95 b	1.18 a	0.91 b	1.01 b	0.069***

[a] Means with different letters in same row differ significantly.
[b] Standard error of mean differences.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

diets. Urinary energy was lower in control diet compared to all treated diets. The urinary energy was 4% of the total DE in the control diet, while on the treated diets, the values ranged from 6 to 9%. Among the treated diets, the molasses treated silage based diet had the highest ME/DE value. The proportion of ME losses as HP was slight lower for the treated silage diets as compared with the control diet. The ME losses as HP among the treated diets were similar and the molasses treated IRG diet lost the lowest (81%) proportion of ME as HP. Among the treated diets, urea treated had the highest ME lost as HP (90%), which was very similar to the control diet. In consequence, the RE of ME was lower for the urea treated IRG based diet (10%) as compared with the other treated silage based diets (Table 5).

3.7. Nitrogen balance

Nitrogen intake and urinary nitrogen (UN) outputs both were higher for the urea treated diet compared to those of the other diets. The amount of N digested was also higher ($P < 0.001$) on the urea treated diet but the diet showed a negative retained N. Retained N per unit of N intake and the retained N per unit of N digested were significantly higher ($P < 0.001$) for the control diet. Among the treated diets the values were higher for molasses and cellulase treated diets. Of the N intake, fecal nitrogen (FN) loss was significantly higher for both urea and molasses treatment, but UN loss was higher for urea treated diet. The loss of UN as a proportion of N intake for all the treated diets were higher as compared with the control diet.

4. Discussion

Methane and nitrogenous compounds excreted by animals are a growing concern as environmental pollutants. Diet can influence production of these compounds. In this study, four different silage additives were examined, where cellulase and cellulase + LAB were the enzyme and inoculant, and molasses and urea are the commonly used additives for upgrading low-quality fibrous feed. In order to evaluate the effects of additives excretion of methane and nitrogen by goats.

4.1. Silage quality

Silages were effectively maintained in drums at room temperature 24°C (21–29°C) during the month

of May to August. This demonstrated that silage can be prepared in metal drum stored at a room temperature of 24°C with no deterioration of the silage. The significant increase in GE of the silage T_2 might be related to the application of urea that formed ammonia, which has a GE content of 91.06 (kcal mol^{-1}) (Givens et al., 1988). The higher lactate and propionate in the silages with lower ratio of acetate: propionate showed the positive effects of molasses, cellulase and cellulase + LAB. Mixing urea increased the pH by formation of ammonia that helped in preservation but produced a higher butyrate and a lower lactate concentration in the silage. The control IRG silage had a higher amount of lactate than urea treated silage but the acetate:propionate was slightly higher as compared with the other silages. The observed values of all the silages were similar to the reported values of IRG silage (Oshima et al., 1991).

Increase in N content was improved by urea treatment but the volatile ammonia N (% of total N) was significantly higher in the silage. The value 57% was much higher than the other silages and the value for well preserved grass silages suggested by Henderson (1993). Molasses mixed silage produced less ammonia N. Yokota et al. (1991) also reported a lower ammonia N (20% versus 10% of the total N) in molasses treated silage. Forage crops suitable for silage should contain an adequate level of fermentative substrate in the form of WSC (McDonald et al., 1991). Italian ryegrass and most of the temperate grasses contained 181 g kg^{-1} (range 74–314) WSC (McDonald et al., 1991), while the IRG harvested at late-bloom stage contained lower WSC than the early bloom stage (JFS, 1999). When 13.3% molasses was mixed with the late-bloom IRG (DM basis), about 62.2 g kg^{-1} of WSC was added to the IRG. Thus, IRG mixed with molasses increased the WSC content that helped preservation.

Cellulase addition resulted in reduction of NDF content of the silage suggesting that the cellulase degraded structural carbohydrate of IRG. However, the cellulase did not improve the digestibility of IRG. Ridla and Uchida (1992) observed a similar response in barley and grass straw silage. The low pH and high lactic acid in the cellulase added silage was due an increase in fermentable carbohydrate (WSC) by hydrolysis of cell wall which causes fermentation by LAB (Ridla and Uchida, 1999). Cellulase may have degraded the most digestible fraction of the structural polysaccharides and left less digestible material (Jacobs and McAllan, 1991) and lowered DM and OM digestibilities of the diet (Table 2). Lactic acid bacteria had no effect on cell wall component, but the cellulase + LAB had a positive effect in reducing silage pH and NH_3-N, and increasing fermentation quality (Cai and Ohmomo, 1995).

4.2. Nutrient utilization

The rumen parameters did not show a large variation among diets except the urea treat silage diet produced a higher ($P < 0.001$) NH_3-N. The observed reductions in total VFA of rumen liquor of control and cellulase diet can be associated with lower OM degradation in the diets. However, the total VFA of the molasses diet showed the potential of mixing molasses in the silage. The observed VFA pattern of the control diet evidenced the effect of concentrate on a little higher propionate proportion in the diet than that of the treated diets. The molasses treated IRG produced higher propionate in the silages but the ratio in rumen liquor was almost similar. The rumen pH values were higher compared to the values of IRG, corn and soybean meal based diets and VFA pattern also similar to that reported by Islam et al. (2000b). However, the rumen pH and VFA concentrations were in the optimum pH and VFA concentration for fiber digestion (Ørskov and Ryle, 1990).

The mean NH_3-N concentration of all diets appeared to have been sufficient to meet the N requirements for rumen microbial population (Ørskov and Ryle, 1990). A significantly higher NH_3-N value for the urea diet (542 mg l^{-1}) was due to the mixing of urea with IRG. A higher (513 mg mean of four samples) NH_3-N was reported by Nishino et al. (1993), where Japanese native goats were fed on Alfalfa silage treated with ammonia. Although they reported no toxic effect and the goats remained healthy, the NRC (1976) reported restlessness muscular tremors and heavy respiration just after introducing the urea treated feed along with a higher rumen NH_3-N concentration. Rumen ammonia can have a direct effect on liver function and peripheral blood ammonia level, can alter the acid–base status, and can change electrolyte balance (Lewis, 1961). Rumen ammonia N concentration of 800 mg l^{-1} would cause

toxicity and can be used as diagnostic tool since blood NH_3-N concentration causing toxicity are difficult to determine (NRC, 1976). Excess rumen ammonia caused frequent urination resulted in a greater urine volume and a higher concentration of N in urine. That might be due to the inability of goats fed on urea based silage diet to use the ammonia generated in the rumen at a higher level than 300 mg l^{-1}. Consequently ammonia was absorbed in the rumen and metabolized into urea in the liver resulting in higher urea N in urine (Yokota et al., 1998).

Higher DM and OM digestibility in the molasses, urea and LAB + cellulase treated IRG increased the digestible nutrients of the diets. Cellulase + LAB did not increase the nutrient digestibility of Rhodes grass, which had less WSC and high structural carbohydrate, and no effect on IRG was found (Ridla and Uchida, 1999), but in the present experiment the combination had higher nutrient digistibilities. Cai and Ohmomo (1995) supported that the cellulase + LAB could increase the DM and OM digestibilities.

The increased urinary volume in urea treated silage diet suggesting that urea treatment increased in urinary nutrient loss. In consequence a lower ME/DE was found in the urea treated diet, which resulted in a lower retained energy compared to the other treatments. The digestibility, metabolizability and ME kg^{-1} DM of the molasses and cellulase + LAB treated diet indicated that the diets were better than either urea or cellulase or control diet. Slightly higher proportions of ME lost as HP for the control diet compared to the treated diets revealed that using the additives prior to ensiling increased in RE proportion of the diet. However, among treatments the urea treatment had a higher ME lost as HP, which resulted in the lower proportion of RE.

The observed amount of CH_4 (g 100 LW^{-1} per day), and MCR in the diets were similar to the recently published data of IRG, corn and soybean meal based diets (Islam et al., 2000b). The calculated CH_4 kg^{-1} DMI data were slightly lower (19.42 g) than that of the mean value of different hay:concentrate diets in goats (Terada et al., 1985). Shibata et al. (1992) showed that CH_4 kg^{-1} DM for sheep and goats was 18.5 and 19.1, respectively, while the value for Holstein heifers was 20.33. Methane kg^{-1} digestible OM, which is one of the suggested parameters for methane inventories from livestock (Moss, 1994) ranged from 32.1 to 35.3 g for the diets. These values were similar with the values obtained from cattle fed different silage based diets with or without fishmeal and soybean meal (Moss, 1994) and were lower compared to the value from beef cattle fed on Angleton grass (75.4 g) and Rhodes grass (64.6 g) diets (Kurihara et al., 1999). However, the CH_4 kg^{-1} OM values (32–35 g) were similar to the value of beef cattle fed high grain diet (32.1 g) (Kurihara et al., 1999). They found no significant variation among the species but reported significant differences among the dietary treatment levels. The CH_4 kg^{-1} OM digested values in dairy cattle, beef cattle and goats showed that species variation of CH_4 emission among ruminants was little but, the variations due to dietary components and feeding system were larger when the parameter was presented as CH_4 kg^{-1} OM digested.

A slightly higher CH_4 was noted in the treated diets as compared with the control diet, but when the value was expressed relative to intake of digestible OM, the treated diets produced lower CH_4. Although the values are non-significant, the proportion of DE lost as CH_4 showed that the IRG treated with the additives prior to ensiling decreased the rate of CH_4 emission. The lower CH_4 emission from the treated IRG may be a result of biochemical factors. In the molasses, cellulase and cellulase + LAB diets, the CH_4 kg^{-1} OM can be explained by the high OM digestibilities of the diets. The treatments have shown to increase the rate of OM degradability and to reduce rumen retention time. The reduced rumen retention time may influence the methanogenic population as methanogens are slow growing and hence proliferate only under condition of slow rumen particle dilution rate (Preston, 1972). The cellulase diet, where lower OM digestibility and lower CH_4 emission was found, might be due to reduced total rumen VFA and reduced acetate concentration (Meeske et al., 1993).

Among diets, the lowest value of CH_4 emission kg^{-1} DOMI (32.1 g) was found in the cellulase + LAB treated diet, where the silage had the lowest NDF content. The lowest CH_4 in the diet may be explained by the fact that the cellulose content of the fibrous component was fermented during ensiling by cellulase, thus lowering fiber degradation in the rumen, which resulted in the lower CH_4. The lower CH_4 values in the molasses and cellulase + LAB diets may have been due to the higher amount of the total

propionate concentration, as increases in propionic acid in rumen liquor reduce the CH_4 production (Ørskov et al., 1991). The overall CH_4 production parameters from IRG based diets were lower than that of corn-included alfalfa based diets in goats (Islam et al., 2000a).

The higher retained N and lower N losses as feces per unit N intake for the control diet were due to a high concentrate level in the diet. Excretion of N in feces and urine follow two different routes with different environmental consequences. With respect to air pollution, Locker and Whitehead (1990) reported that volatilization of ammonia from urinary N was at least 5–6 times higher than from fecal N. Thus, sifting of N excretion from urine to feces was suggested by Castillo et al. (2000). In the soil, 24% of urinary N leached to below a depth of 150 mm in non-irrigated fields with the remaining urinary N in the soil converted from urea to ammonium within 24 h (Pakrou and Dillon, 1995).

The molasses, cellulase and cellulase + LAB had similar urinary losses, but the fecal N loss was highest in the molasses diet and it had slight positive N balance. This showed that shifting of N excretion from urine to feces could be possible using molasses based diet. In tropical environments, the general situation is low CP diets and urea is used to increase the dietary CP. The higher ($P < 0.001$) UN loss in the urea treated diet resulted in a lower retained N per unit N intake and retained N per unit N digested and finally no improvement of N retention. Therefore, urea treatment (4% of the IRG DM) resulted in an increase in the leaching NO_3-N from animal source to surface, ground and drinking water. Finally the excess N may cause environmental pollution as ammonia and nitrate in the manure (Yano and Nakajima, 1996). A higher urinary loss was also found in an alfalfa only (100%) diet where dietary N was significantly higher (Islam et al., 2000a). This supports our earlier statement that the dietary N requirement must be clearly estimated to reduce N excretion through feces and urine (Islam et al., 2000b).

5. Conclusion

This study showed that Italian ryegrass mixed with cellulase prior to ensiling showed decreased methane emission when fed to goats. Combined treatment of cellulase and LAB improved silage digestibility, and energy and N utilization by goats. Using urea increased the urinary energy and nitrogen losses, resulted in lower retained energy and nitrogen. However, using molasses prior to ensiling showed positive effects on energy and N with a lower urinary nitrogen excretion. Decreasing the N excretion through urine and possibility of shifting it to fecal N could also be obtained using molasses. Balancing of energy and protein in diets will increase feed efficiency, decrease nitrogen excretion and reduce environmental pollutants like CH_4 and N. Therefore, it is possible not only to reduce methane emission from goats, but at the same time to increase total animal protein output.

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