Asian-Aust. J. Anim. Sci.
Vol. 20, No. 6 : 887 - 893
June 2007

www.ajas.info

Influence of Varying Dry Matter and Molasses Levels on Berseem and Lucerne Silage Characteristics and Their *In situ* Digestion Kinetics in *Nili* Buffalo Bulls

N. A. Touqir[1], M. Ajmal Khan[1,2,] *, M. Sarwar[1], M. Nisa[1], W. S. Lee[2], H. J. Lee[2] and H. S. Kim[2]

[1]Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, 38000, Pakistan

ABSTRACT : Influences of forage DM and addition of cane molasses on silage characteristics of berseem (*Trifolium alexandrium*) and lucerne (*Medicago sativa*) and their ruminal digestion kinetics in *Nili* buffaloes were studied. Berseem and lucerne fodders (at one tenth bloom) were ensiled with wheat straw in laboratory silos to achieve 20, 30, 40% forage DM and without wheat straw (control); each forage DM level was supplemented with 2, 4 and 6% of cane molasses at ensiling. The pH and lactic acid contents of berseem and lucerne silages were affected by both forage DM and addition of molasses. Dry matter, CP and true protein (TP) of berseem and lucerne silages were affected by forage DM at ensiling but were not affected by the addition of cane molasses. Higher DM, CP and TP losses were observed when berseem and lucerne fodders were ensiled either without wheat straw or with wheat straw to achieve 20% and 40% forage DM at ensiling compared with 30% DM at ensiling. Fiber fractions (NDF, ADF, hemicellulose and cellulose) of berseem silage and lucerne silage were significantly increased with increasing forage DM at ensiling. Addition of cane molasses did not affect the DM, CP, TP and fiber fractions of both berseem and lucerne silages. Berseem and lucerne ensiled at 30% DM with 2% cane molasses were screened for comparative ruminal digestion kinetics with their respective fodders. Addition of wheat straw to berseem or lucerne fodder at ensiling depressed DM and NDF ruminal degradability. However, ruminal lag time, rate of degradation and extent of digestion of silages were similar to their respective fodders. In conclusion, berseem and lucerne could be ensiled with wheat straw to increase their DM to 30% along with 2% molasses for buffaloes. (**Key Words** : Berseem, Lucerne, Silage, Legume, Buffalo, Digestion)

INTRODUCTION

Cost effective milk and meat production requires regular supply of good quality forage. In south Asia, low and irregular supply of forages coupled with diminution in area under fodder cultivation is the main constraint, which is adversely affecting ruminant productivity (Khan et al., 2004, 2006a, b, c). Climatic extremes primarily limit fodder production in south Asian region and thus affect the regular fodder availability to ruminants. Constant supply of forage could be achieved by ensiling when the fodders are abundantly available (Sarwar et al., 2006). However, few attempts have been made to ensile locally available fodders (Touqir et al., 2007) and scientific information on the nutritive value of both grass and legume silages are also scarce in buffaloes.

Lucerne (*Medicago sativa*) and berseem (*Trifolium alexandrium*) are multi-cut, high yielding leguminous perennial fodders in the region and can be ensiled to fulfill the forage demand during lean periods of fodder availability (Sarwar et al., 2005; Khan et al., 2006b). Preservation of fodder is achieved by acid production leading to steady decline in pH under anaerobic condition. Fermentable carbohydrate, nitrogen, DM contents, type and amount of bacterial population on the forage at ensiling time were important factors that affected its buffering capacity during ensiling and thus silage quality (Khorasani et al., 1993; Khan et al., 2006b; Nisa et al., 2005). However, leguminous fodders have high buffering capacity due to high moisture and protein contents. Therefore, pH drops slowly during ensiling and resulted in higher nutrient losses (Bolsen, et al., 1996). Ensiling high moisture crops could result in clostridial fermentation that led to heavy loss of nutrients (Gary, 1992; Matsuoka et al., 1993). Therefore, before ensiling the lucerne or berseem, moisture contents should be reduced either by field wilting or by the addition of some absorbent. Berseem was wilted before ensiling to lower its moisture contents (Fransen and Strubi, 1998) but wilting is laborious and costly.

* Corresponding Author: Muhammad Ajmal Khan. Tel: +82-41-580-3398, Fax: +82-41-580-3419, E-mail: ajmals1@yahoo.com
[2] Dairy Cattle Research Division, National Livestock Research Institute, Korea.
Received May 15, 2006; Accepted February 7, 2007

Table 1. Chemical composition (Mean±SD) of berseem lucerne, and wheat straw on DM basis

Parameters (%)	Berseem[1]	Lucerne[1]	Wheat straw
Dry matter	17.2±1.80	17.0±2.10	91.7±1.4
Crude protein	17.1±1.34	18.3±1.61	3.22±0.44
True protein	13.9±1.12	13.7±1.44	DN[2]
NDF	47.1±2.20	48.0±2.21	75.1±2.31
ADF	29.8±1.48	30.5±1.92	49.0±2.22
Hemicellulose	17.5±1.23	18.2±1.44	25.4±2.76
Cellulose	22.5±1.59	23.2±1.62	39.42±2.84
Acid detergent lignin	7.00±0.56	8.00±0.42	8.2±0.32
Ash	11.4±0.66	10.8±0.54	7.0±0.41

[1] Berseem and lucerne fodders were harvested at one-tenth bloom stage for laboratory scale silage preparation.
[2] DN = Did not determined.

Dry roughages (wheat or rice straws) have high DM and low nitrogen (N) contents and thus can be used to reduce the moisture content of berseem and lucerne before ensiling (Khan et al., 2006a, b). However, since these fodders have higher protein and minerals contents and low fermentable carbohydrates (Bolsen et al., 1996, Nisa et al., 2006a, b), cane molasses may be used as an additive to improve fermentable sugars and to promote the growth of epiphytic lactic acid bacteria for their better preservation. However, scientific information on chemical composition and nutritive value of berseem and lucerne ensiled at different DM and molasses levels is limited.

Therefore this study examined the effect of various DM and cane molasses levels on berseem and lucerne silage characteristics, chemical composition and their *in situ* digestion kinetics in *Nili* buffalo bulls (*Bubalus bubalis*).

MATERIALS AND METHODS

In vitro experiments

Fodders : Berseem and lucerne seeds were procured from Nuclear Institute of Agriculture and Biology, Faisalabad and were sown in the fields adjacent to Animal Nutrition Research Center, University of Agriculture Faisalabad, Pakistan. Berseem and lucerne fodders were harvested at one-tenth bloom stage for silage preparation at laboratory scale. Berseem and lucerne fodder samples (n = 10 per fodder) were chopped in a locally manufactured chopper. These samples were dried at 55°C and ground to 2mm particle size through a Wiley mill for subsequent chemical analyses.

The DM of berseem and lucerne samples was determined by drying them at 105°C for 4 h followed by equilibration in a desiccator (AOAC, 1999; ID 930.5), and organic matter (OM) was calculated as weight loss upon ignition at 600°C. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents in both fodders were determined with the ANKOM fiber analyzer using reagents described by Van Soest et al. (1991). The N contents of both fodders were determined by a Kjeldahl method (AOAC 1999; ID 984.13). Chemical composition of berseem and lucerne fodder harvested at one-tenth bloom for ensiling is given in Table 1.

Table 2. Chemical composition (mean±SD) of cane molasses (% DM)

Parameters	Cane molasses
Dry matter	66.0±2.10
Crude protein	4.50±0.21
Ash	10.7±2.77
Dextrose	1.3±0.14
Sucrose	37.0±2.10
Fructose	4.1±0.23

Preparation of laboratory silos : Berseem and lucerne fodders were chopped (5 cm) with a locally manufactured chopper for ensiling. Each fodder was ensiled with wheat straw (chemical composition is given in Table 1) to achieve 20, 30, 40% DM contents at the time of ensiling and without wheat straw (control) in laboratory silos (transparent thick polyethylene bags of 10-kg capacity having dimensions (80×40 cm)). At the time of ensiling, cane molasses were added to each treatment (DM level of berseem and lucerne) at the rate of 2, 4 and 6% of forage DM. Chemical composition of molasses is given in Table 2. Four silos per treatment were prepared and placed at the room temperature (25°C) for 30 days.

After opening these silos, silage pH was recorded using a pH-mV meter (HM-21P, TOA Corporation, Tokyo, Japan). Lactic acid in samples was determined in aqueous extracts by means of a GLC with a semi-capillary FFAP (nitroterephthalic acid-modified polyethylene glycol) column (Hewlet-Packard, Wardbronn, Germany), over a temperature range of 45 to 230°C. True protein (TP) was estimated by multiplying TCA insoluble-N×6.25. Berseem silage and lucerne silage samples were analyzed for DM, N, NDF, ADF, cellulose, ADL, and total ash using method described above.

Statistical analysis : The data generated during laboratory trials on silage characteristics and chemical composition of berseem and lucerne were separately analyzed using analysis of variance technique each according to factorial arrangement of treatments (4×3) i.e. three levels of additive (2, 4 and 6%) and four levels of DM (control, 20, 30 and 40%) to determine best combination of DM content and additive level (SAS, 1994). The statistical model used for all parameters was;

$$Y_{ijk} = \mu+\alpha_j+\beta_k+(\alpha\beta)_{jk}+\varepsilon_{ijk}$$

Where, μ was overall mean.

α_j was the effect of additive levels (2, 4 and 6%),

β_k was the effect of DM contents (20, 30, 40% and control),

$(\alpha\beta)_{jk}$ was the interactions between additive level and DM level

ε_{ijk} was the difference within treatment means (error term).

The significant ($p<0.05$) differences among treatment means were separated by Duncan's Multiple Range test was applied (Steel and Torrie, 1984).

Nylon bag experiments

Large scale ensiling : Berseem and lucerne fodders (one-tenth bloom stage) and their best-screened silages (30% DM with 2% molasses level) were used to determine comparative *in situ* DM and NDF digestion kinetics. Berseem and lucerne fodders were chopped (5 cm) using locally manufactured chopper for large scale ensiling. Each fodder was ensiled separately with wheat straw to achieve 30% DM along with 2% molasses of forage DM for 30 days in cemented trench silos (8×4×2 m). Cemented trench silos were filled with chopped fodder and pressed properly to remove air for attaining good anaerobiasis. Each silo was covered with 10 cm thick layer of rice straw, followed by covering with a plastic sheet. The plastic sheet was then plastered with a blend of wheat straw and mud (5 cm thick) to avoid any cracking while drying. It was presumed that plastic sheet and mud plastering provided anaerobic condition for proper silage making. Berseem silage and lucerne silage samples were analyzed for pH, lactic acid, DM, N, TP, NDF, ADF, cellulose, ADL, and total ash using method described above. Chemical composition of berseem and lucerne ensiled in cemented trench silos is presented in Table 5.

Animals and feeding : Two *in situ* experiments were conducted to evaluate ruminal digestion kinetics of berseem fodder and lucerne fodder with their silages.

In each experiment 6 ruminally cannulated buffalo bull were used. The buffalo bulls were housed on a concrete floor in separate pens. Fifteen days were given as adaptation period to the diet at the start of each experiment followed by 4 days of ruminal incubation of *in situ* nylon bags. In first experiment, three bulls were fed berseem fodder based diet and other threes were given berseem silage based diet. Similarly in second experiment, three bulls were fed lucerne fodder based diet while other three were given lucerne silage. The fodders and silages contributed 80% of the dietary DM in both experiments the remaining 20% DM in each diet was supplied by cotton seed meal (10%) and wheat bran (10%). Ruminally cannulated buffalo bulls were fed the same diet as was being incubated in their rumen to avoid the effects of diet on the ruminal fermentation pattern (Clark and David, 1990).

In each experiment, nylon bags measuring 13×21 cm, with an average pore size of 50 μm, were used to study *in situ* digestion kinetics. For each time point, 10 g DM of each sample (berseem fodder, lucerne fodder and/or their silages) was weighed into bags, in triplicate. In each bull, two bags were used to determine DM and NDF disappearance and the third bag served as a blank (having no sample). The bags were closed and tied with nylon fishing line and were exposed to ruminal fermentation for 1, 2, 4, 6, 10, 16, 24, 36, 48, 72, and 96 h. After removal from the rumen, bags were washed in running tap water (15 minutes) until the rinse was clear. Bags were then dried in oven at 55°C for 48 h. After equilibration with air for 8 h, the bags were weighed and the residues were transferred to 100 ml cups and analysed for DM and NDF as described above. Ruminal DM and NDF digestion, rate of digestion and lag time, were determined for each incubation time.

Table 3. Influence of different levels of forage dry matter and cane molasses on chemical composition of berseem silage

Parameters	Forage dry matter (%)												SE	Main effects		Interaction
	Control (17.2)			20			30			40						
	Molasses levels (%)			Molasses levels (%)			Molasses levels (%)			Molasses levels (%)						
	2	4	6	2	4	6	2	4	6	2	4	6		A	B	A×B
pH	4.65	4.51	4.50	4.55	4.43	4.39	4.12	4.08	4.02	4.35	4.32	4.30	0.16	–	+	+
Lactic acid	3.00	3.00	3.25	3.40	3.45	3.54	3.94	3.95	3.90	3.60	3.64	3.62	0.28	–	+	+
Dry matter	16.0	14.5	14.4	18.3	18.1	17.6	29.8	28.5	27.2	38.5	36.6	36.6	2.56	--	NS	NS
Crude protein	13.9	14.6	13.8	14.5	13.5	13.5	15.2	15.4	14.5	13.6	13.6	12.6	0.75	--	NS	NS
True protein	9.6	9.2	9.0	10.3	10.3	10.5	11.9	12.4	12.2	9.2	9.8	10.1	0.69	--	NS	NS
NDF	43.6	43.5	42.8	45.5	44.5	44.4	50.7	49.3	48.7	55.7	54.7	54.5	3.71	--	NS	NS
ADF	27.6	27.2	27.0	27.8	27.2	27.0	31.6	31.2	31.2	34.5	34.4	34.4	2.66	--	NS	NS
Hemicellulose	16.0	16.3	15.8	17.6	16.3	17.4	19.1	18.1	17.5	21.2	20.3	20.2	1.66	--	NS	NS
Cellulose	21.2	20.9	20.7	21.4	20.9	20.8	24.1	23.9	24.1	26.2	26.2	26.3	2.46	--	NS	NS
ADL	7.90	7.99	7.87	7.47	7.30	7.21	7.16	7.30	7.12	9.32	9.25	9.09	1.33	--	NS	NS
Ash	11.2	11.4	11.4	11.3	11.4	11.4	10.9	11.0	11.1	10.3	10.4	10.6	0.14	NS	NS	NS

– = Significant ($p<0.05$), –+ = Significant ($p<0.01$), NS = Non-significant, A = Dry matter levels, B = Molasses levels.

Berseem fodder (one-tenth bloom stage) was ensiled with wheat straw to achieve 20, 30, and 40% DM contents at the time of ensiling and without wheat straw (control) in laboratory silos at room temperature (25°C) for 30 days. Cane molasses were added to each treatment (DM level of berseem) at the rate of 2, 4 and 6% of forage DM.

Table 4. Influence of different levels of forage dry matter and cane molasses on chemical composition of lucerne silage

Parameters	Fodder dry matter (%)												SE	Main effects		Interaction
	Control (17.0%)			20%			30%			40%						
	Molasses levels (%)			Molasses levels (%)			Molasses levels (%)			Molasses levels (%)						
	2	4	6	2%	4	6	2	4	6	2	4	6		A	B	A×B
pH	4.65	4.60	4.50	4.55	4.51	4.48	4.03	4.00	3.91	4.15	4.20	4.20	0.57	+	+	−
Lactic acid	3.00	3.08	3.32	3.38	3.43	3.50	3.72	3.78	3.84	3.43	3.49	3.58	0.19	++	+	−
Dry matter	15.6	15.3	15.6	19.7	18.6	18.7	28.6	29.8	28.2	37.4	37.7	38.6	0.63	++	NS	NS
Crude protein	14.3	15.0	16.1	16.8	17.0	16.0	14.8	14.5	14.5	12.9	12.8	12.8	0.62	++	NS	NS
True protein	9.0	9.0	9.4	10.0	10.4	10.3	11.7	12.0	12.6	8.9	8.8	9.1	0.69	++	NS	NS
NDF	45.3	45.0	44.9	45.4	45.0	44.9	50.4	49.9	49.8	55.5	54.9	54.7	0.50	++	NS	NS
ADF	28.6	28.3	28.0	29.0	28.8	28.9	32.4	31.7	31.9	35.3	35.4	35.3	0.55	++	NS	NS
Hemicellulose	16.7	16.8	16.9	16.5	16.7	16.7	18.4	18.2	18.4	20.2	19.5	19.4	0.41	++	NS	NS
Cellulose	21.2	21.0	20.8	21.4	21.1	21.0	23.6	23.5	23.9	26.1	26.4	26.9	0.34	++	NS	NS
ADL	7.89	7.78	7.67	7.45	7.58	7.17	8.29	8.11	8.94	9.40	9.13	9.36	0.28	+	NS	NS
Ash	10.9	11.0	11.0	10.9	11.0	11.1	10.5	10.6	10.8	10.0	10.1	10.2	0.06	NS	NS	NS

− = Significant ($p<0.05$), −+ = Significant ($p<0.01$). NS = Non-significant, A = Dry matter levels, B = Molasses levels.

Lucerne fodder (one-tenth bloom stage) was ensiled with wheat straw to achieve 20, 30, and 40% DM contents at the time of ensiling and without wheat straw (control) in laboratory silos at room temperature (25°C) for 30 days. Cane molasses were added to each treatment (DM level of berseem) at the rate of 2, 4 and 6% of forage DM.

Table 5. Chemical composition (mean±SD) of berseem silage and lucerne silage ensiled in cemented trench silos for feeding and nylon bag study

Parameters	Berseem silage[1]	Lucerne silage[1]
pH	4.18±0.13	4.31±0.24
Lactic acid	3.98±0.14	3.79±0.20
Dry matter	28.4±2.11	27.9±1.93
Crude protein	15.0±1.38	14.5±1.45
True protein	11.2±0.54	10.32±0.34
NDF	50.0±2.41	49.6±2.51
ADF	31.0±2.66	30.4±2.32
Hemicellulose	18.4±1.87	17.7±2.42
Cellulose	23.2±1.98	21.8±1.87
ADL	7.98±1.22	8.90±1.45
Ash	10.4±1.20	11.1±1.33

[1] Berseem and lucerne fodders were harvested at one-tenth bloom stage. Each fodder was ensiled separately with wheat straw to achieve 30% DM along with 2% molasses of forage DM for 30 days in cemented trench silos.

individually. Degradation rates were determined by subtracting the indigestible residue, i.e. the 96 h of ruminal incubation, from the amount in the bag at each time point and then regressing the natural logarithm of that value against time (Sarwar et al., 2004) after correcting for lag time (Mertens, 1977). The lag time was calculated according to Mertens and Loften (1980).

Statistical analysis : The data (lag time, rate and extent of digestion of DM and NDF) from two ruminal digestion kinetics studies, separately on berseem and lucerne, were analyzed according to Student's *t*-test (SAS, 1994).

RESULTS AND DISCUSSION

In vitro experiments

Chemical composition of berseem fodder and lucerne fodders harvested at one tenth bloom stage for ensiling is presented in Table 1.

The pH and lactic acid contents of berseem silage were affected ($p<0.05$) by both forage DM and addition of molasses at ensiling time (Table 3). The interaction between forage DM and molasses levels were also significant ($p>0.5$) for both pH and lactic acid contents of berseem silage. Similar results were noticed when lucerne fodder was ensiled at different DM levels with cane molasses (Table 4). These results were consistent with the findings of Yunus et al. (2000) who reported that addition of molasses to fodder before ensilation lowered silage pH due to increased lactic acid production. Addition of corn starch or molasses to Mott grass (*Pennisitum purpureum*) at ensiling has improved the availability of fermentable sugars for anaerobic fermentation that lead to higher acid production and thus lower silage pH (Nisa et al., 2005). Higher lactic acid content and corresponding lower pH of oat grass ensiled with cane molasses were attributed to the availability of easily fermentable sugars for better growth of lactic acid producing bacteria (Bureenok et al., 2005; Sarwar et al., 2006, Touqir et al., 2007). This higher production of lactic acid lowers the silage pH and terminates the microbial activity in the ensiled material needed for its preservation (McDonald et al., 1991; Bolsen et al., 1996). However, Leibensperger and Pitt (1998) reported higher fermentable sugars of un-wilted forages with the addition of molasses did not always reduce the final silage pH at very low (<20%) DM. Moisture is one of the important determinants of pH decline during ensiling. Lower moisture at ensiling retarded the growth of anaerobic bacteria and thus reduces the conversion of easily fermentable carbohydrates to organic acids. Higher moisture in ensiling crops diluted the amount of acid produced and thus resists the pH decline during ensiling (Khan et al., 2006b, c). Lower pH and higher lactic acid

Table 6. Comparative *in situ* dry matter and neutral detergent fiber digestion kinetics of berseem fodder and its silage

Parameters	Berseem fodder[1]	Berseem silage[2]	SE
Dry matter			
Degradability[3] (%)	68.76[a]	65.91[b]	1.31
Lag (hour)	1.93	1.97	0.18
Rate of degradation (%/h)	4.24	4.19	0.12
Extent of digestion[4] (%)	72.86	71.71	3.41
Neutral detergent fiber			
Degradability[3] (%)	57.1[a]	54.8[b]	0.30
Lag (h)	2.11	2.03	0.12
Rate of degradation (%/h)	3.86	3.64	0.29
Extent of digestion[4] (%)	62.07	61.85	4.30

[1] Berseem fodder was harvested at one-tenth bloom stage.
[2] Berseem fodder was ensiled with wheat straw to achieve 30% DM along with 2% molasses of forage DM for 30 days in cemented trench silos (8×4×2 m).
[3] Degradability was determined at 48 h of ruminal incubation.
[4] Extent of digestion was determined at 96 hours of ruminal incubation
Means within row bearing different superscripts differ significantly ($p<0.05$).
SE: Standard error between means.

contents were observed when berseem and lucerne fodders were ensiled at 30% DM level compared with control, 20 and 40% DM levels.

Dry matter contents of berseem and lucerne silages are affected ($p<0.01$) by forage DM at ensiling time. However, the addition of cane molasses at ensiling time did not affect the berseem silage and lucerne silage DM. The interaction between forage DM content and molasses levels was not significant for DM content of berseem silage (Table 3) and lucerne silage (Table 4). In present study, the addition of wheat straw has improved the DM of both berseem and lucerne fodders at ensiling, while addition of molasses has improved fermentable sugar contents of the ensiled material. Thus the associative effect of wheat straw and molasses might have improved DM recovery of berseem and lucerne silages by reducing its loss. Previously, Yunus et al. (2000) and Man and Wiktorsson (2001) reported that use of molasses as silage additive led to reduce the loss of nutrients by early pH decline and stabilization of the medium. Our previous study (Khan et al., 2006b) indicated that higher DM and fiber fractions in oat grass ensiled with molasses were because of the reduced activity of plant and microbial enzymes because of early drop in pH and early stability of the medium compared with oat grass ensiled without molasses. Similar results were reported by McDonald et al. (1991), Bolsen et al. (1996) and Yunus et al. (2000). In present experiment better silage nutrient composition was noticed when berseem and lucerne fodders were ensiled with wheat straw to achieve 30% forage DM along with 2% cane molasses.

Crude protein and TP contents of berseem silage and lucerne silage were affected ($p<0.01$) by the forage DM at ensiling, but those were not affected by the addition of cane molasses. The interaction between forage DM and addition of cane molasses were also noticed non-significant for CP and TP contents in both berseem and lucerne silages. Higher CP and TP loss was noticed when berseem and lucerne fodders were ensiled without wheat straw or with wheat straw to achieve 20% and 40% forage DM at ensiling compared with 30% DM at ensiling. Other researchers (Khorasani et al., 1993) described that in very high (>45%) and very low (<20%) DM silages pH decline was not rapid and fermentation was prolonged that led to extensive proteolysis and nutrient losses. Previously, Kung et al. (2000) reported an extensive proteolytic activity by microbes and plant proteolytic enzymes that resulted in loss of CP contents to NH_3-N in grass silage. In present study the variation in CP content of silages was because of the differences in the CP contents of the ensiled material that may be attributed to supplementation of fodder with wheat straw to adjust different DM levels before ensiling.

Fiber fractions (NDF, ADF, hemicellulose and cellulose) and ADL of berseem and lucerne silages were significantly ($p<0.01$) influenced by forage DM at ensiling. However, the effect of cane molasses and the interaction between forage DM and molasses levels at ensiling were noticed non-significant for these parameters (Tables 3 and 4). Neutral detergent fiber, ADF, hemicellulose and cellulose in berseem and lucerne silages were increased with increasing forage DM at ensiling because of the addition of wheat straw. Several workers (Sheperd et al., 1995; Sheperd and Kung, 1996; Cone et al., 1999; Kung et al., 2000; Nadeau et al., 2000) previously reported decreased fiber contents of silages which were associated with proteolytic and fibrolytic activities of microbes and plant enzymes. These plant and microbial enzymes are acid labile with optimum pH ranging between 5 to 6 and their activity become negligible as pH approached 5 (McDonald, 1981). Higher DM, protein and fiber fractions in berseem and lucerne silages ensiled with wheat straw and cane molasses were probably because of the reduced activity of plant and microbial enzymes due to their low pH (3.91-4.65). Khan et al. (2006b) reported that acid hydrolysis of fiber fraction and excessive losses of nutrients due to prolonged fermentation increased the lignin contents of silage. Higher lignin contents in grass were also reported when grass was supplemented with wheat straw at ensiling (Nisa et al., 2005, 2006b). However, in the present study, both lucerne and berseem have comparable lignin contents to that of wheat straw, thus it's ensiling with berseem and lucerne fodders did not change the silage ADL contents.

Nylon bag experiments

Ruminal DM and NDF degradability (at 48 h) of berseem fodder was higher ($p<0.05$) than its silage. Ruminal lag time, rate of degradation and extent of digestion of DM and NDF were noticed similar for berseem

Table 7. Comparative *in situ* dry matter and neutral detergent fiber digestion kinetics of lucerne fodder and its silage

Parameters	Lucerne fodder[1]	Lucerne silage[2]	SE
Dry matter			
Degradability[3] (%)	70.27[a]	68.12[b]	1.31
Lag (h)	1.94	1.91	0.11
Rate of degradation (%/h)	4.27	4.19	0.42
Extent of digestion[4] (%)	72.60	72.92	3.35
Neutral detergent fiber			
Degradability[3] (%)	58.03[a]	55.91[b]	1.02
Lag (h)	2.18	2.09	0.21
Rate of degradation, (%/h)	3.69	3.73	0.32
Extent of digestion[4] (%)	63.41	64.27	5.31

[1] Lucerne fodder was harvested at one-tenth bloom stage.
[2] Lucerne fodder was ensiled with wheat straw to achieve 30% DM along with 2% molasses of forage DM for 30 days in cemented trench silos.
[3] Degradability was determined at 48 h of ruminal incubation.
[4] Extent of digestion was determined at 96 hours of ruminal incubation
Means within row bearing different superscripts differ significantly ($p<0.05$).
SE: Standard error between means.

fodder and its silage (Table 6). Similar results were noticed when ruminal degradability of lucerne was compared with its silage in buffalo bulls (Table 7). In contrast to present results. Nadeau et al. (1996) reported that ensiling increased the ruminal DM and NDF degradability due to fermentative decomposition of cell wall components of fodder. However, in concurrence to present results. reduced ruminal degradability of silage compared to its fodder was reported (Rook and Thomas, 1982; Ruiz et al., 1992) and attributed to the extensive loss of soluble carbohydrates due to microbial fermentation during ensiling (Sarwar et al., 2005, Khan et al., 2006c). In present study wheat straw was added to both berseem and lucerne fodders to adjust their DM (30%) at ensiling. The addition of less degradable wheat straw (Khan et al., 2006b) to berseem and lucerne fodders has probably resulted in depressed DM and NDF digestibility of their silages. Further the loss of fermentable carbohydrates during ensiling (Nisa et al., 2005; Touqir et al., 2007) might have depressed the ruminal degradability of berseem and lucerne silages compared to fodders.

CONCLUSIONS

Berseem and lucerne fodders ensiled with wheat straw to increase their DM 30% along with 2% molasses have shown better silage characteristics and nutrients recovery. The addition of wheat straw to berseem and lucerne fodders at ensiling has depressed the ruminal degradability (at 48 h) however, ruminal lag time, rate of degradation and extent of digestion were noticed similar for fodders and their silages in *Nili* buffalo bulls. Further research is warranted to examine the effects of berseem and lucerne silages in the diets of lactating buffaloes.

REFERENCES

AOAC. 1999. Official Methods of Analysis (16th Ed). Official Methods of Analysis of AOAC International, Gaithersburg, MD, USA.

Bolsen, K. K., G. Ashbell and Z. G. Weinberg. 1996. Silage fermentation and silage additives: Review. Asian-Aust. J. Anim. Sci. 9:483-489.

Bureenok, S., T. Namihira, M. Tamaki, S. Mizumachi, Y. Kawamoto and T. Nakada. 2005. Fermentative quality of Guinea grass silage by using fermented juice of the epiphytic lactic acid bacteria (FJLB) as a silage additive. Asian-Aust. J. Anim. Sci. 18:807-813.

Clark, J. H. and C. L. David. 1990. Some aspects of feeding high producing dairy cows. J. Dairy Sci. 68:873-881.

Cone, J. W., A. H. V. Gelder, I. A. Soliman, H. De Visser and A. M. Van Vuuren. 1999. Different techniques to study rumen fermentation characteristics of maturing grass and grass silage. J. Dairy Sci. 82:957-964.

Fransen, S. C. and F. J. Strubi. 1998. Relationships among absorbents on the reduction of grass silage effluent and silage quality. J. Dairy Sci. 81:2623-2629.

Gary, M. 1992. Ensiling Process. In: Silage Manual (Ed. M. Bjorge and H. Bjorge). Edmonton, Alberta. pp. 14-17.

Khan, M. A., M. Sarwar, M. Nisa and M. S. Khan. 2004. Feeding value of urea treated corncobs ensiled with or without enzose (corn dextrose) for lactating crossbred cows. Asian-Aust. J. Anim. Sci. 17:1093-1097.

Khan, M. A., M. Sarwar, M. Nisa, S. A. Bhatti, Z. Iqbal, W. S. Lee, H. J. Lee, H. S. Kim and K. S. Ki. 2006a. Feeding value of urea treated wheat straw ensiled with or without acidified molasses in *Nili-Ravi* buffaloes. Asian-Aust. J. Anim. Sci. 19:645-650.

Khan, M. A., M. Sarwar, M. Nisa, M. S. Khan, Z. Iqbal, W. S. Lee, H. J. Lee and H. S. Kim. 2006b. Chemical composition, *in situ* digestion kinetics and feeding value of Oat grass (*Avena sativa*) ensiled with molasses for *Nili-Ravi* Buffaloes. Asian-Aust. J. Anim. Sci. 19:1127-1133.

Khan, M. A., M. Sarwar, M. Nisa, S. A. Bhatti, Z. Iqbal, W. S. Lee, H. J. Lee, H. S. Kim and K. S. Ki. 2006c. Feeding value of urea treated wheat straw ensiled with or without acidified molasses in *Nili-Ravi* buffaloes. Asian-Aust. J. Anim. Sci. 19:645-650.

Khorasani, G. R., E. K. Okine, J. J. Kennelly and J. H. Helm. 1993. Effect of whole crop cereal grain silage substituted for alfalfa silage on performance of lactating dairy cows. J. Dairy Sci. 76:3536.

Kung, Jr., L. J. R. Robinson, N. K. Ranjit, J. H. Chen, C. M. Golt and C. D. Pesek. 2000. Microbial populations, fermentation end products and aerobic stability of corn silage treated with ammonia or a propionic acid-based preservative. J. Dairy Sci. 83:1479-1486.

Leibensperger, R. Y. and R. E. Pitt. 1998. Modeling the effects of formic acid and molasses on ensilage. J. Dairy Sci. 71:1220-1228.

Man, N. V. and H. Wiktorsson. 2001. Cassava tops ensiled with or without molasses as additive effect on quality, feed intake and digestibility by heifers. Asian-Aust. J. Anim. Sci. 14:624-631.

Matsuoka, S., S. Yonezawa, H. Ishitabi, K. Osanai and H. Fujita.

1993. The effect of moisture content on aerobic deterioration of grass silage. Proc. World Conf. Anim. Prod. Edmonton, Canada. pp. 100-101.

McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. Biochemistry of Silage. (2nd Ed.), Chalcombe Publications, Marlow, UK. p. 184.

Mertens, D. R. 1977. Dietary fiber components: relationship to the rate and extent of ruminal digestion. Feed Process. 36:187-199.

Mertens, D. R. and J. R. Loften. 1980. The effect of starch on forage fiber digestion kinetics *in vitro*. J. Dairy Sci. 63:1437-1444.

Nadeau, E. M. G., D. R. Buxton, E. Lindgren and P. Lingvall. 1996. Kinetics of cell-wall digestion of orchard grass and alfalfa silages treated with cellulase and formic acid. J. Dairy Sci. 79:2207-2014.

Nadeau, E. M. G., D. R. Buxton, J. R. Russell, M. J. Allison and J. W. Young. 2000. Enzyme, bacterial inoculant and formic acid effects on silage composition of orchard grass and alfalfa. J. Dairy Sci. 83:1487-1492.

Nisa, M., N. A. Touqir, M. Sarwar, M. A. Khan and M. Akhatar. 2005. Effect of additives and fermentation periods on chemical composition and *in situ* digestion kinetics of Mott grass (*Pennisitum purpureum*) silage. Asian-Aust. J. Anim. Sci. 18:812-815.

Nisa, M., M. A. Khan, M. Sarwar and M. Mushtaque. 2006a. Influence of re-growth interval on chemical composition, herbage yield, digestibility and digestion kinetics of *Setaria sphacelata* and *Cenchrus ciliaris* in Buffaloes. Asian-Aust. J. Anim. Sci. 19:381-385.

Nisa, M., M. A. Khan, M. Sarwar, W. S. Lee, H. J. Lee, S. B. Kim, B. S. Ahn and H. S. Kim. 2006b. Influence of corn steep liquor on feeding value of urea treated wheat straw in buffaloes fed at restricted diets. Asian-Aust. J. Anim. Sci. 19:1610-1616.

Rook, J. A. F. and P. C. Thomas. 1982. Silage for milk production. National Institute of Research in Dairying, Reading, England. ISBN 0-7084-0166-X.

Ruiz, T. M., W. K. Sanchez, C. R. Straples and L. E. Sollenberger. 1992. Comparison of "Mott" dwarf elephant grass silage and corn silage for lactating dairy cows. J. Dairy Sci. 75:533-539.

Sarwar, M., M. A. Khan and M. Nisa. 2004. Influence of ruminally protected fat and urea treated corncobs ensiled with or without corn steep liquor on nutrient intake, digestibility, milk yield and its composition in *Nili-Ravi* buffaloes. Asian-Aust. J. Anim. Sci. 17:86-93.

Sarwar, M., M. A. Khan, M. Nisa and N. A.Touqir. 2005. Influence of berseem and lucerne silages on feed intake, nutrient digestibility and milk yield in lactating *Nili* buffaloes. Asian-Aust. J. Anim. Sci. 18:475-478.

Sarwar, M., M. Nisa, M. A. Khan and M. Mushtaque. 2006. Chemical composition, herbage yield, and nutritive value of *Panicum antidotale* and *Pennisetum orientale* for *Nili* buffaloes at different clipping intervals. Asian-Aust. J. Anim. Sci. 19:176-184.

SAS (Statistical Analysis System). 1994. 'SAS user's guide: Statistics' (SAS Inst. Inc., Carry, NC).

Sheperd, A. C., M. Maslanka, D. Quinn and L. Kung. 1995. Additives containing bacteria and enzymes for alfalfa silage. J. Dairy Sci. 78:565-572.

Sheperd, A. C. and L. Kung, Jr. 1996. Effect of an enzyme additive on composition of corn silage ensiled at various stages of maturity. J. Dairy Sci. 79:1767-1774.

Steel, R. G. D. and J. H. Torrie. 1984. Principles and Procedures of Statistics. A Biometrical Approach. (2nd Ed.) McGraw Hill Book Co. Inc. New York, USA.

Touqir, N. A., M. A. Khan, M. Sarwar, M. Nisa, W. S. Lee, K. S. Ki, H. J. Lee and H. S. Kim. 2007. Feeding value of Jambo grass silage and Mott grass silage for lactating *Nili* buffaloes. Asian-Aust. J. Anim. Sci. 20:523-528.

Van Soest, P. J., H. B. Robertson and B. A. Lewis. 1991. Method of dietary fiber and non-starch polysaccharides in relation to animal material. J. Dairy Sci. 74:3583-3591.

Yunus, M., N. Ohba, M. Furuse and Y. Masuda. 2000. Effects of adding urea and molasses on napier grass silage quality. Asian-Aust. J. Anim. Sci. 13:1542-1547.