

Evaluation of Structural Carbohydrates Losses and Digestibility in Alfalfa and Orchardgrass during Ensiling 1

M. S. Yahaya*, A. Kimura, J. Harai, H. V. Nguyen, M. Kawai, J. Takahashi and S. Matsuoka

Department of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan

ABSTRACT : The evaluation of structural carbohydrate losses and its effect on silages digestibility in alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) was studied during 5, 21 and 56 days ensiling. About 70 and 60 kg fresh matter of the two forages were ensiled in 9 silos of 120 L capacity. The digestion trials were conducted in two phases using the two grasses in two 4 × 4 Latin square design according to the four treatments being the grass and the three silages. There were no differences in the DM and CP contents resulting from 5 to 56 days ensiling in both forages. The water-soluble carbohydrates (WSC), hemicellulose, pectin, and energy were slightly reduced and appeared lower in 56 days silage. The ether extract and cellulose contents slightly increased as the ensiling process advanced in the two species. Hemicellulose losses of 29 and 41 g/kg DM were obtained in alfalfa and orchardgrass, respectively, 56 days after ensiling. While the cellulose losses in both species were very little, compared to that for hemicellulose, the pectin losses, 56 days after ensiling were 15 and 12 g/kg DM in alfalfa and orchardgrass respectively. The total structural carbohydrates lost (ie., hemicellulose + cellulose + pectin) in g/kg DM of fresh material forage ensiled, is about four fifths the amount lost by WSC, in alfalfa and about two thirds, in orchardgrass, by 21 days ensiling after the activity of microorganism terminated, indicating that appreciable amount was used as substrate for silage fermentation. Ensiling alfalfa and orchardgrass for 0, 5, 21 and 56 days maintained a decreasing trend of 83.8, 82.5, 79.3 and 78.9% digestibility in alfalfa and 80.5, 77.0, 77.1 and 76.4% digestibility in orchardgrass. While the digestibility of cellulose and ether extract increased in silage in both species, the digestible energy values in silage were reduced from 2.6 to 2.3 and 2.9 to 2.7 Mcal/kg DM respectively in alfalfa and orchard during 5-56 days ensiling. (*Asian-Aust. J. Anim. Sci.* 2001. Vol 14, No. 12 : 1701-1704)

Key Words : Alfalfa, Orchardgrass, Ensiling, Structural Carbohydrates, Digestibility

INTRODUCTION

There is a lot of conflicting information on the change of carbohydrate component of grasses and legumes during ensiling (Michael, 1961). Previous reports indicate that the acids produced during ensiling are those from the fermentation of the water soluble carbohydrates (WSC) alone (McDonald and Whittenbury, 1977). Later reports suggested that other substances can also act as substrates (McDonald et al., 1991). During ensiling the breakdown of the carbohydrates component depends on factors that are complex and not fully understood.

When forage is ensiled, two to three weeks are required to attain the stable stage. Before this stage two to three inter-related stages, of association take place: (1) Plant cell respiration - aerobic microbial active stage (2) lactic acid bacterial active stage and then the stable stage. It is assumed that stages 1 and 1-2 are completed within 3 and 21 days of ensiling respectively (McDonald et al., 1991). The stable stage (3) depends on the overall nature of fermentation of the ensiled crop.

Ensiling alfalfa and orchardgrass for 5, 21 and 56 days would provide an assessment of the amount of changes in the nutrients composition during these three stages. This

study was carried out to examine the extent of structural carbohydrates losses in the (two fodder) legume and grass as material forage and to determine the effects of the losses on the nutritive value of the produced silage.

MATERIALS AND METHODS

Silage preparation

The alfalfa (*Medicago sativa*) and orchardgrass (*Dactylis glomerata*) were collected from the University farm around June. About 70 kg and 60 kg fresh matter from alfalfa and orchardgrass, respectively, was wilted and chopped into a length of 3-5 cm. This were then mixed, and randomly ensiled into nine (9) plastic silos of 120 liters capacity. Three silos were opened at the end of 5, 21, and 56 days of ensiling and weighed to determine the extent of structural carbohydrates losses in each silo. A representative sample from each silo was mixed, sub-sampled and the three silos mixed and stored at -15°C for the digestion trial.

Digestibility trial

The digestion trial was conducted in two phases using the two grasses. During each phase feeds were offered to four castrated sheep at the rate of 50 g/kg⁻¹ BW^{0.75} (body weight per day), fed twice daily in a 4 × 4 Latin square design according to the four treatments being the grass and the three silages. The Periods of measurement consisted of

* Address reprint request to M. S. Yahaya. Tel: +81-155-49-5423, Fax: +81-155-49-5462, E-mail: Saniyahaya@hotmail.com
Received April 19, 2001; Accepted June 14, 2001

12 days, the first 7 days were for adaptation and the last 5 days for collection. Water and minerals (mineral contained Fe 1,232; Cu 25; Zn 500; I 50; Se15; and Na 382 mg/kg) were provided at all time (*ad libitum*).

Chemical analyses

The DM content of grass and silage was determined by freeze drying method. The crude protein (CP), ether extract (EE) and gross energy were determined by standard procedures (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as described by Goering and Van soest (1970) as modified by Van soest et al. (1991). Cellulose and hemicellulose contents were calculated by subtracting ADL from ADF and ADF from NDF respectively. Water-soluble carbohydrates (WSC) and pectin were determined according to methods of Deriaz (1961) and Taylor and Buchanan-Smith (1992), respectively. Total digestible nutrient (TDN) and digestible crude energy were calculated according to method of Banerjee (1988).

Statistical analyses

Data on structural carbohydrates losses were analyzed using ANOVA in a randomized block design, while digestibility data were subjected to ANOVA of 4 × 4 Latin square design with means difference determined using multiple range test procedures (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The chemical composition of the two forages and their silages are shown in table 1. The WSC contents in both forage materials were lowered during ensiling and extensively decreased with the advance of the stage of ensiling in orchardgrass. The hemicellulose and pectin contents trended to be slightly reduced over the ensiling periods in both species. However the change in cellulose values were small compared to those for hemicellulose and pectin in the both species evaluated.

Losses of WSC and structural carbohydrates during ensiling

The WSC are the main substrate for silage fermentation and almost all are lost during ensiling. In this study, about 50 and 83 g/kg DM of WSC were lost from alfalfa and orchardgrass, respectively, after 56 days ensiling, and the greatest quantity was lost within 5 days of ensiling (table 2).

Previous studies indicated that there was a wide range of variation in hemicellulose loss during ensiling, as being 11.4 to 54.4% (McDonald et al., 1960, 1962; Butler and Bailey, 1973; McDonald et al., 1991). Although no possible reason for this wide variation was evident, this variation can

Table 1. Chemical Composition (%) of the two forages and subsequent silage

	Length of ensiling			
	0 day	5 days	21 days	56 days
Alfalfa				
Dry matter	28.9	28.4	28.3	28.2
Crude protein ¹	17.6	16.6	16.5	16.0
Ether extract	2.4	2.9	3.4	3.7
Water-soluble carbohydrate	6.2	1.6	1.4	1.3
Hemicellulose	46.40	14.4	13.0	12.3
Cellulose	22.1	21.5	21.7	22.2
Pectin	8.7	8.2	8.1	7.4
Energy (Mcal/kg DM)	4.01	3.92	3.86	3.84
Orchardgrass				
Dry matter	31.4	31.0	30.6	30.5
Crude protein ¹	10.9	11.0	11.1	10.9
Ether extract	2.9	3.7	3.9	4.0
Water-soluble carbohydrate	9.5	4.4	1.7	1.2
Hemicellulose	23.7	22.8	21.0	20.5
Cellulose	29.5	29.8	29.7	30.5
Pectin	6.2	6.0	5.7	5.6
Energy (Mcal/kg DM)	4.37	4.24	4.21	4.23

¹ Dry matter basis

be partially explained by differences in the length of the ensiling period, the forage species and the fermentation quality of silage. In this study, 29 and 41 g/kg DM of hemicellulose losses were obtained in alfalfa and orchardgrass, respectively, 56 days after ensiling. Dewar et al. (1963) reported three possible ways of hemicellulose decomposition during ensiling. These are (1) actions of hemicellulases present in the original forage (2) facultative anaerobic bacterial action and (3) hydrolysis by organic acids produced during fermentation.

Hemicellulose losses during 0 to 5, 5 to 21 and 21 to 56 days of ensiling periods were highest for 5 to 21 days, being about half of the total loss obtained from 0 to 56 days. The loss during this period is considered to be due to action of anaerobic bacteria and hydrolysis by organic acids (McDonald et al., 1960). However, this may play an insignificant role because the loss during 21-56 days was the lowest, although this period was longest, suggesting that the loss due to hydrolysis by organic acids was small. The loss value for 0 to 5 days period was substantially low compared to that for 5 to 21 days period, and the loss is considered to be due to action of hemicellulase present in the original forage and actions of aerobic and facultative anaerobic bacteria.

In contrast the cellulose losses in both species were very

Table 2. Losses of Water soluble carbohydrate and structural carbohydrates (g/kg DM) during ensiling

	Length of ensiling			SE
	5 days	21 days	56 days	
	Lucerne (g/kg DM) ¹			
Water-soluble carbohydrate	46	49	50	<1
Structural carbohydrates	27 ^c	40 ^b	51 ^a	<1
total				
Hemicellulose	8 ^c	22 ^b	29 ^a	2
Cellulose	12	10	7	2
Pectin	7 ^b	8 ^b	15 ^a	<1
	Orchardgrass (g/kg DM) ¹			
Water-soluble carbohydrate	53 ^b	79 ^a	83 ^a	<1
Structural carbohydrates	25 ^b	54 ^a	55 ^a	<1
total				
Hemicellulose	15 ^b	36 ^a	41 ^a	3
Cellulose	3 ^b	8 ^a	2 ^b	<1
Pectin	7 ^b	10 ^a	12 ^a	<1

^{a,b,c} Means followed by the different superscripts differ ($p < 0.05$).

¹ Each values indicated means of three silos.

small, compared to that for hemicellulose. This is consistent with earlier work by Morrison (1979). The pectin losses, 56 days after ensiling were similar in both species, showing 15 to 12 g/kg DM, approximately half of it was lost during 0 to 5 days ensiling.

Losses in gram per kilogram DM of harvested material forage ensiled, the amount lost by structural carbohydrates (hemicellulose + cellulose + pectin) is about four fifths the amount lost by WSC, in alfalfa and about two thirds, in orchard-grass, by 21 days ensiling after the activity of microorganism terminated. This reveals that appreciable amounts of structural carbohydrates are used as substrate, although in general it is considered that the main substrates for silage fermentation are WSC.

Apparent digestibility and nutritive value of fresh material and silages

The apparent nutrient digestibility and nutritive value of alfalfa and orchardgrass and silages are shown in table 3. There was no difference ($p > 0.05$) in DM digestibility between the grass and the subsequent silage in the two species evaluated. It was well established that when crops are ensiled in a well sealed silo the digestibility of both DM and organic matter of the silage are similar to those in the crop before ensilage (Wilkins, 1981). The ether extract digestibility values were higher in the subsequent silage compared to the material forage (grass) in both species possibly from the increased contents of organic acids, particularly VFA and lactic acid, which are reported to be increased during fermentation (Dewar et al., 1963). In contrast, the digestibility of energy values in alfalfa were higher ($p < 0.05$) in the material forage than in the

Table 3. Apparent digestibility and nutritive value of fresh material and silages

	Length of ensiling				SE
	0 day	5 days	21 days	56 days	
	Alfalfa				
Digestibility (%)					
Hemicellulose	83.8 ^a	82.5 ^{ab}	79.3 ^b	78.9 ^b	0.85
Cellulose	67.9 ^b	67.2 ^b	70.4 ^a	70.3 ^a	0.47
Nutritive value (%)					
TDN (%)	63.9	62.5	63.3	63.9	0.16
DCP (%)	12.7 ^a	11.6 ^b	11.5 ^b	11.1 ^c	0.04
DE (Mcal/kg DM)	2.7 ^a	2.4 ^b	2.4 ^b	2.3 ^c	0.01
	Orchardgrass				
Digestibility (%)					
Hemicellulose	80.5	77	77.1	76.40	0.84
Cellulose	79.1	79.2	80.1	78.70	0.66
Nutritive value (%)					
TDN (%)	63.3	65.5	66.5	65.7	0.69
DCP (%)	6.6	6.8	7.0	6.5	0.16
DE (Mcal/kg DM)	2.9	2.8	2.7	2.7	0.04

^{a,b,c} Means within each row having different superscript letter differ significantly ($p < 0.05$).

subsequent silage resulting from the combined losses of other nutrients such as DM, CP, WSC, hemicellulose, cellulose and pectin during ensiling. The energy digestibility appeared lowest during 56 days ensiling in both species even though it was not significant in orchardgrass. This could be a result of more material being used for respiration by silage microorganism when ensiled for longer than for shorter periods (Mchan, 1986) leaving more material resistant to digestion.

The hemicellulose digestibility during 0, 5, 21 and 56 days ensiling maintained a decreasing trend of 83.8, 82.5, 79.3, 78.9% in alfalfa ($p < 0.05$) and 80.5, 77.0, 77.1 and 76.4%, in the orchard-grass (table 2). These decreasing trends of hemicellulose digestibility could be due to the increased losses of hemicellulose fractions across the lengths of ensiling in the two species (table 3). During fermentation the hemicellulose is rapidly hydrolyzed to monosaccharides fractions which are readily WSC (Dewar et al., 1963). Morrison (1979) observed that the decrease in hemicellulose soluble fractions was proportional to the increase in fiber fractions, leaving indigestible fractions in the silage and thus reduces its digestibility. Other reasons for silage lower digestibility could be due to differences accruing from the breakdown of the hemicellulose components during fermentation (Boever et al., 1983). Earlier work by McDonald et al. (1960) in a number of experiments determined hemicelluloses of herbage and silages as galactan + araban + Zylan and their losses appeared higher in araban (52.7%), followed by galactan

(29.2%) and Zylan (24.8%) during fermentation. Similarly, Daughtry et al. (1978) reported higher losses of 74.7% arbinose compared to 53.7% for zylose during *in vitro* fermentation. This indicates zylan to be consistently less digestible and more abundant than araban (Daughtry et al., 1978). It is possible that the reduced digestibility of silage in this study might be due to higher content of zylose fractions in silages as reflected by the higher hemicellulose losses.

Cellulose digestibility increased ($p < 0.05$) as the ensiling advanced in alfalfa due to the actions of extra-cellular cellulase from silage microflora, which are capable of shortening the cellulose chains lengths making it more accessible to enzymatic attack (Morrison, 1979, 1988; Galligan and Reese, 1954). In orchardgrass the cellulose digestibility appeared about the same throughout the length of ensiling. McDonald et al. (1962) reported no difference in the cellulose digestibility between grass material and its silage.

CONCLUSION

This study indicates that there were no differences in the contents of DM and CP resulting from 5 to 56 days ensiling in both species. The same trend was observed in DM digestibility. While the WSC, hemicellulose, pectin, and energy were slightly reduced and appeared lower in 56 days silage, the ether extract and cellulose contents slightly increased as the ensiling advanced in the two species. The digestible energy values in silage were reduced in both forages with advance stages of ensiling. The total lost of structural carbohydrate (i.e., pectin+hemicellulose+cellulose) indicates that appreciable amount of these components were used as substrate for silage fermentation.

REFERENCES

- AOAC. 1990. Official Methods of Analysis, 15th ed. Assoc. of Official Anal. Chem. Washington, DC. pp. 1235-1241.
- Banergee, G. C. 1988. Computation of digestible nutrients and TDN. In: Feeds and Principles of Animal Nutrition Revised Edition Oxford & IBH Publ. (Ed. G. C. Banergee). CO.PTV.LTD New Delhi. pp. 556-559.
- Boever, J. L. De, J. V. Aerts, B. G. Cottyn, D. L. Brabander and F. X. Buysse. 1983. Changes in digestibility and nutritive value of maize with stage of maturity. *Rev. Agric.*, 36, 263-271.
- Butler, G. W. and R. W. Bailey. 1973. Criteria for assessing the efficiency of the fermentation process. In: Chemistry and Biochemistry of Herbage Butler (Ed. G. W. Bailey, R. W.) 3:33-80.
- Daughtry, C. S. T., D. A. Holt and V. L. Echtenberg. 1978. Concentration, composition, and *in vitro* disappearance of hemicellulose in tall fescue and orchard-grass. *Agron. J.* 62:550-554.
- Deriaz, R. E. 1961. Routine analysis of carbohydrate and lignin in herbage. *J. Sci. of Food and Agric.* 12:152-160.
- Dewar, W. A., P. McDonald and R. Whittenbury. 1963. The hydrolysis of grass hemicelluloses during ensilage. *J. Sci. Food Agric.* 14:411-417.
- Galligan, W. and E. T. Reese. 1954. Evidence of for multiple components in microbial cellulases. *Canadian Analytical Chemistry* 28, 350-356.
- Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis Apparatus, Reagents, Procedures and Some applications. *Agric. Handbook* 379. ARSUSDA. Washington, DC.
- McDonald, P., A. R. Henderson and S. J. E. Hero. 1991. In: The Biochemistry of Silage Chalcombe Publications, 2nd Ed. (Ed. P. McDonald, A. R. Henderson, S. J. E. Heron). pp. 9-340
- McDonald, P. and R. Whittenbury. 1977. Chemistry and biochemistry of herbage (Ed. G. W. Butler and R. W. Bailey). Academic press, New York. 3:33-36.
- McDonald, P., A. C. Sterling, A. R. Henderson, W. A. Dewar, G. H. Stark, H. T. Macpherson, A. M. Reid and J. Slater. 1960. Studies on ensilage. *Edin. Sch. Agric. Tech. Bul.*, 24:1-83.
- McDonald, P., A. C. Stirling, A. R. Henderson and R. Whittenbury. 1962. Fermentation studies in wet herbage. *J. Sci. Food Agric.* 13:581-590.
- Mchan, F. 1986. Cellulose treated coastal bermudagrass silage and production of soluble carbohydrates, silage acids and digestibility *J. Dairy Sci.* 69:431-438.
- Michael K. W. 1961. The silage fermentation (Ed. I. L. Allen and I. M. Richard). Marcel Dekker, Inc. New York pp. 1-22.
- Morrison, I. M. 1988. Influence of some chemical and biological additives on the fiber fraction of lucerne on ensilage in laboratory silo. *J. Agric. Sci.* 111:35-39.
- Morrison, I. M. 1979. Changes in the cell wall components of laboratory silages and the effect of various additives on these changes. *J. Agric. Sci., Camb.* 93:581-586.
- Snedecor, G. W. and W. G. Cochran. 1980. Two-way classifications and analysis of variance In: Statistical Methods (Ed. G. W. Snedecor and W. G. Cochran). 7th ed. Iowa state university press pp. 255-269.
- Taylor, K. A. and Buchanan-Smith, 1992. A calorimetric method for the quantitation of uronic acids and a specific assay for galacturonic acid. *Analytical biochem.* 201:190-196.
- Van soest, P. J., J. B. Robertson and B. A. Lewins. 1991. Methods of dietary fibre, neutral detergent fiber and non starch Polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 55: 805-810.
- Wilkins, R. J. 1981. The nutritive value of silage In: Recent Developments in Ruminant Nutrition (Ed. W. Haresign and D. J. A. Cole). Butterworths publications 2nd Ed. pp. 268-283.