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# Effect of length of ensiling on silo degradation and digestibility of structural carbohydrates of lucerne and orchardgrass

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**Abstract**

This study determined the extent of structural carbohydrate loss from lucerne (*Medicogo sativa* L.) and orchardgrass (*Dactylis glomerata* L.) after 5, 21 and 56 days of ensiling. About 70 and 60 kg fresh matter of lucerne and orchardgrass, respectively, were ensiled in nine silos of 120 l capacity. For each forage, the four treatments (i.e. fresh material and 5, 21 and 56 days silages) were fed to four male castrated sheep in a $4 \times 4$ Latin square design experiment to determine effects of ensiling on digestibility. The overall fermentation quality of the two forages was judged to be good and within acceptable guidelines. Dry matter (DM) and gross energy losses were small, confirming an acceptable fermentation process. Hemicellulose losses of 19.8 and 17.2% occurred in lucerne and orchardgrass, respectively, by 56 days of ensiling. Cellulose losses in both forages were small compared to those of hemicellulose. Pectin loss by 56 days of ensiling were similar to hemicellulose, being 17.3% in lucerne and 17.7% in orchardgrass. Hemicellulose digestibility decreased ($P < 0.05$) in lucerne and orchardgrass as ensiling advanced, while cellulose digestibility was higher ($P < 0.05$) in days 21 and 56 silages than in the harvested lucerne grass. An appreciable amount of structural carbohydrate (i.e. pectin + hemicellulose + cellulose) were degraded during fermentation. 

*Keywords:* Ensiling; Lucerne; Orchardgrass; Structural carbohydrates; Digestibility

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## 1. Introduction

Information on degradation of cellulose, hemicelluose, and pectin in silage during fermentation is scarce relative to that available for protein and non-structural carbohydrates (Michael, 1984). Similarly, little is known of the utilization of these components as microbial substrates during ensiling. While previous reports indicate that the bulk of the acids produced during ensiling originate from fermentation of water-soluble carbohydrates (WSC) (McDonald and Whittenbury, 1977), more recent reports suggest that substances such as protein and structural carbohydrates can also be substrates (McDonald et al., 1991). Cellulose, hemicellulose and pectin may also be substrates for microorganisms during fermentation, and their degradation during ensiling depends on interrelated factors, such as differences in forage species, forage growth stage at harvest, moisture content at ensiling and period and length of ensiling of forage, which are not fully understood.

When forage is ensiled, 2–3 weeks are generally required for it to attain stability (McDonald et al., 1991). The length of time that silage is stable due to a low pH depends on the nature of the fermentation in the ensiled crop.

This study examined the extent of degradation of structural carbohydrates of lucerne and orchardgrass after 5, 21 and 56 days of ensiling, as well as effects of silage structural carbohydrate degradation on in vivo digestibility in sheep.

## 2. Materials and methods

### 2.1. Silage preparation

Lucerne (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) were harvested during the early flowering and heading stage, respectively, at the Obihiro University Farm, Japan. The two species were wilted for 8 h and chopped into lengths of 3–5 cm using a mechanical forage cutter. The two species were mixed individually and representative samples obtained. About 70 kg fresh matter of lucerne and 60 kg fresh matter of orchardgrass from the remaining content of each species were ensiled in nine plastic silos of 120 l capacity. Three silos from each species were opened after 5, 21 and 56 days of ensiling and weighed to determine the extent of structural carbohydrate loss. A representative sample from each silo was mixed and sub-sampled. The remaining content of the three silos was mixed and frozen at $-15^{\circ}$C for the subsequent digestion trial.

### 2.2. Digestibility trial

Digestion trials were conducted separately for the two forages. For each forage trial, the four treatments (i.e. harvested material and silages after 5, 21 and 56 days) were fed to four male castrated sheep in a $4 \times 4$ Latin square design experiment. Sheep were fed twice daily at a maintenance energy level (50 g dry matter (DM)/(kg body weight (BW)$^{0.75}$) at 07:30 and 17:30 h after orts had been removed, weighed and sub-sampled. Water and minerals (mineral block from Nihonzenyaku Limited, Tokyo) were provided ad libitum. The mineral block contained: Fe 1232; Cu 150; Co 25; Zn 500; Mn 500; I 50; Se 15; and

Na 382 mg/kg. Each period of the trial consisted of 7 days of adaptation to the respective forage and 5 days of recording of feed intake and fecal output.

### 2.3. Chemical analyses

The DM content of harvested material and resultant silage from the two species were determined by freeze drying for a minimum of 24 h. The crude protein (CP) and ether extract (EE) were determined using Kjeldahl and Soxlet apparatus, respectively, as described by procedures 954.01 and 954.02, respectively (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as described by Goering and Van Soest (1970) as modified by Van Soest et al. (1991) without the use of sodium sulfite and amylase. Acid detergent lignin (ADL) was determined using 72% $H_2SO_4$ solution as described by Goering and Van Soest (1970) as modified by Van Soest et al. (1991). Cellulose and hemicellulose were calculated by subtracting ADL from ADF and ADF from NDF, respectively. Silage pH was immediately determined from the prepared silages extract using a pH meter probe. Standard procedures were applied to estimate WSC (Deriaz, 1961), pectin (Taylor and Buchnan-Smith, 1992), lactic acid (Baker and Summerson, 1961), volatile fatty acids (VFA; gas chromatography according to George and Melvin, 1979), and ammonia (Conway and O'Malley, 1942). The energy content of feed and faeces were determined directly by bomb calorimetry (adiabatic bomb calorimeter CA-4P, Shimadzu, Tokyo).

### 2.4. Statistical analyses

Silage fermentation data were analyzed using ANOVA in a randomized block design, while data obtained from the digestibility trial were analyzed as a $4 \times 4$ Latin square design, with means differences determined using a multiple range test (Duncan, 1955).

## 3. Results and discussion

### 3.1. Composition of the fresh material and silages

The WSC contents of all silages declined ($P < 0.05$) as the length of ensiling increased (Table 1). The hemicellulose and pectin contents decreased during ensiling in both species ($P < 0.05$), while cellulose contents in both forages increased ($P < 0.05$).

The pH values in both forages were lower ($P < 0.05$) at 21 and 56 days of fermentation compared to 5 days, while the lactic acid concentration was higher (Table 2). The acetic acid content decreased ($P < 0.05$) in lucerne, but increased in ochardgrass, as ensiling advanced. Similar changes in pH, associated with increases in lactic acid, were observed in normal silages made from lucerne and orchardgrass (Butler and Bailey, 1973; Morrison, 1988). The silage obtained from 56 days fermentation of both forages were judged to be good (i.e. within acceptable guidelines) as specified by Butler and Bailey (1973) and Chamberlain and Wilkinson (1996). However, the butyric acid content in orchardgrass

Table 1
Chemical composition (g/kg DM) of harvested forages and silages[a]

| | Length of ensiling (days) | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 21 | 56 | S.E. |
| Lucerne | | | | | |
| Dry matter (g/kg) | 289 | 284 | 283 | 282 | 0.9 |
| Crude protein | 176 | 166 a | 165 a | 160 b | 6.3 |
| Ether extract | 24 | 29 a | 34 b | 37 c | 0.7 |
| Water-soluble carbohydrate | 62 | 16 a | 14 b | 13 c | 1.0 |
| Neutral detergent fiber | 464 | 455 a | 444 b | 144 b | 1.2 |
| Acid detergent fiber | 316 | 311 a | 314 b | 321 c | 1.5 |
| Acid detergent lignin | 96 | 96 c | 97 b | 99 a | 0.9 |
| Hemicellulose | 148 | 144 a | 129 b | 123 c | 1.4 |
| Cellulose | 221 | 215 a | 217 a | 222 c | 2.0 |
| Pectin | 87 | 82 a | 81 a | 74 c | 1.4 |
| Gross energy (MJ/kg DM) | 17 | 16 | 16 | 16 | 0.1 |
| Orchardgrass | | | | | |
| Dry matter | 314 | 310 a | 306 b | 305 b | 2.2 |
| Crude protein | 109 | 110 a | 111 a | 109 b | 1.5 |
| Ether extract | 29 | 37 a | 39 b | 40 b | 1.4 |
| Water-soluble carbohydrate | 95 | 44 a | 17 b | 12 c | 0.6 |
| Neutral detergent fiber | 558 | 553 a | 534 b | 536 b | 4.3 |
| Acid detergent fiber | 321 | 325 a | 324 a | 331 c | 2.2 |
| Acid detergent lignin | 26 | 27 a | 27 a | 26 b | 0.5 |
| Hemicellulose | 237 | 228 a | 210 a | 205 b | 3.1 |
| Cellulose | 295 | 298 b | 297 b | 305 a | 2.8 |
| Pectin | 62 | 60 a | 57 b | 56 c | 0.7 |
| Gross energy (MJ/kg DM) | 18 | 18 | 18 | 18 | 0.1 |

[a] Values are means of three silos except for fresh material; means followed by different letters within the same row differ ($P < 0.05$).

silage was intermediate as judged from guidelines specified by Chamberlain and Wilkinson (1996) and McDonald et al. (1991).

### 3.2. Losses during ensiling

The DM losses at any stage of fermentation (Table 3) were within the acceptable range of 2–4% suggested by McDonald et al. (1991). Similarly the gross energy losses in both forages by 56 days of ensiling were 7.5 and 7.0%, respectively, and approximately equal to the 6% unavoidable energy loss due to residual respiration by plant enzymes and fermentation by microorganisms (Zimmer, 1980).

The WSC are the main substrate for silage fermentation and almost all are gradually lost during ensiling. In this study, about 80 and 88% of WSC were lost in lucerne and orchardgrass, respectively, after 56 days of ensiling, and the greatest quantity was lost within 5 days of ensiling.

Previous studies indicate that there is a wide variation (i.e. 11.4–54.4%) in hemicellulose loss during ensiling (McDonald et al., 1960, 1962, 1991; Butler and Bailey, 1973), and

Table 2
Fermentation characteristics (g/kg DM) of lucerne and orchardgrass silages[a]

| | Length of ensiling (days) | | | |
|---|---|---|---|---|
| | 5 | 21 | 56 | S.E. |
| Lucerne | | | | |
| pH | 5.03 a | 4.34 b | 4.19 b | 0.1 |
| Lactic acid | 26 a | 54 b | 64 b | 6 |
| Acetic acid | 12 a | 8 b | 4 c | <1 |
| Propionic acid | – | – | – | – |
| Butyric acid | 2.0 | – | – | – |
| Ammonia (g/kg total N) | 44 a | 51 b | 54 b | <1 |
| Orchardgrass | | | | |
| pH | 4.72 a | 4.34 b | 4.19 c | 0.1 |
| Lactic acid | 25 a | 44 b | 53 c | 4 |
| Acetic acid | 5 a | 7 ab | 10 b | <1 |
| Propionic acid | – | – | – | – |
| Butyric acid | 1 a | 2 a | 3 b | 0 |
| Ammonia (g/kg total N) | 48 a | 66 b | 80 c | 5 |

[a] Values are means of three silos; means followed by different letters within the same row differ ($P < 0.05$).

no clear reason for this wide variation has been suggested. In this study, 19.8 and 17.2% of hemicellulose was lost in lucerne and orchardgrass, respectively, by 56 days of ensiling. Dewar et al. (1963) suggests three mechanisms of hemicellulose decomposition during ensiling: (1) action of hemicellulases present in the original forage; (2)

Table 3
Losses (%) of DM, gross energy, water-soluble carbohydrate and structural carbohydrates in lucerne and orchardgrass[a]

| | Length of ensiling (days) | | | |
|---|---|---|---|---|
| | 5 | 21 | 56 | S.E. |
| Lucerne | | | | |
| Dry matter | 2.6 | 3.1 | 3.5 | 0.3 |
| Gross energy | 4.8 | 6.6 | 7.5 | 0.7 |
| Water-soluble carbohydrate | 74.3 | 78.3 | 79.9 | 1.6 |
| Hemicellulose | 5.1 a | 15.2 b | 19.8 c | 1.0 |
| Cellulose | 5.4 | 4.6 | 3.3 | 0.9 |
| Pectin | 8.3 a | 9.7 a | 17.3 b | 1.6 |
| Orchardgrass | | | | |
| Dry matter | 2.2 | 3.4 | 3.9 | 0.5 |
| Gross energy | 5.1 a | 7.1 b | 7.0 b | 0.4 |
| Water-soluble carbohydrate | 55.3 a | 82.6 b | 88.0 c | 5.1 |
| Hemicellulose | 6.3 a | 14.8 b | 17.2 b | 1.8 |
| Cellulose | 0.9 a | 2.5 b | 0.5 a | 0.4 |
| Pectin | 10.2 a | 15.7 b | 17.7 b | 0.9 |

[a] Values are means of three silos; means followed by different letters within silages within the same row differ ($P < 0.05$).

facultative anaerobic bacterial action, and (3) hydrolysis by organic acids produced during fermentation.

Cellulose losses in both forages were small when compared with those for hemicellulose, consistent with Morrison (1979). Pectin losses increased ($P < 0.05$) as ensiling advanced in both forages, the losses reaching 17.3% in lucerne and 17.7% in orchardgrass by days 56. There is very little information available on pectin losses during ensilage, although, pectin losses were 17.9, 16.7 and 11.7% in orchardgrass, timothy, and lucerne, respectively, at 35 days of ensiling (Mastsuoka et al., 1997).

### 3.3. Apparent digestibility of fresh material and silages

There were no differences ($P > 0.05$) in DM digestibility between the harvested forage and the subsequent silage within the two species (Table 4). It is well established that crops ensiled in properly sealed silos have digestibilities of DM and OM very similar to that in the crop before ensiling (Wilkins, 1981).

Hemicellulose digestibility in lucerne decreased ($P < 0.05$) as ensiling advanced. A similar trend was observed in orchardgrass. Digestion studies (Morrison, 1979; Daughtry et al., 1978; McDonald et al., 1960) of hemicellulose components have shown araban to be consistently digestible. The decrease in hemicellulose digestibility of silages compared to the fresh forage may have been due to the readily digestible fraction of hemicellulose araban that was degraded during ensilage. However, in lucerne, digestibility of cellulose was higher ($P < 0.05$) in the days 21 and 56 silages than in days 5 silage and harvested

Table 4
Apparent nutrient digestibility (g/kg DM) of harvested material and silages[a]

| | Length of ensiling (days) | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 21 | 56 | S.E. |
| Lucerne | | | | | |
| Dry matter | 671 | 662 | 673 | 672 | 2 |
| Crude protein | 722 a | 698 b | 701 b | 691 b | 3 |
| Ether extract | 758 | 785 | 789 | 768 | 12 |
| Gross energy (MJ/kg DM) | 639 a | 621 b | 626 b | 591 c | 2 |
| Structural carbohydrates | | | | | |
| Hemicellulose | 838 a | 825 ab | 793 b | 789 b | 9 |
| Cellulose | 679 a | 672 a | 704 b | 703 b | 5 |
| Orchardgrass | | | | | |
| Dry matter | 675 | 679 | 689 | 669 | 7 |
| Crude protein | 605 | 617 | 628 | 595 | 14 |
| Ether extract | 446 a | 592 b | 610 b | 599 b | 16 |
| Gross energy (MJ/kg DM) | 655 | 651 | 658 | 637 | 7 |
| Structural carbohydrates | | | | | |
| Hemicellulose | 807 | 770 | 771 | 764 | 8 |
| Cellulose | 791 | 792 | 801 | 787 | 7 |

[a] Values are means of four sheep; means followed by different letters within the same row differ ($P < 0.05$).

forage. This increase of cellulose digestibility may be a result of shortening of the cellulose chain length by the action of extra-cellular cellulases from silage microflora, resulting in it being more susceptible to enzymatic attack (Galligan and Reese, 1954; Morrison, 1979, 1988).

## 4. Conclusion

The DM digestibility of lucerne and orchardgrass were not influenced by ensiling, however, losses of hemicellulose and pectin increased as ensiling advanced. Hemicellulose digestibility decreased in the lucerne and orchardgrass as ensiling advanced probably due to losses of these components during fermentation. Cellulose digestibility was higher in days 21 and 56 silages as a result of extra-cellular cellulase activity from silage microflora.

## References

Association of Official Analytical Chemists (AOAC), 1990. Assoc. of Official Anal. Chem., 15th Edition. Washington, DC, pp. 69–89.

Baker, S.B., Summerson, W.H., 1961. The calorimetric determination of lactic acid in biological material. J. Biol. Chem. 138, 535–554.

Butler, G.W., Bailey, R.W., 1973. Criteria for assessing the efficiency of the fermentation process. In: Butler, G.W., Bailey, R.W. (Eds.), Chemistry and Biochemistry of Herbage, Vol. 3. pp. 33–80.

Chamberlain, A.T., Wilkinson, J.M., 1996. The ideal silage. In: Chamberlain, A.T., Wilkinson, J.M. (Eds.), Feeding the Dairy Cow. Chalcombe Publications, Lincol, UK, pp. 28–30.

Conway, E.J., O'Malley, E., 1942. Microdiffussion methods: ammonia and urea using buffer absorbents (revised methods for ranges greater than 10 (g N). Biochem. J. 36, 655–661.

Daughtry, C.S.T., Holt, D.A., Lechtenberg, V.L., 1978. Concentration, composition, and in vitro disappearance of hemicellulose in tall fescue and orchardgrass. Agron. J. 62, 550–554.

Deriaz, R.E., 1961. Routine analysis of carbohydrate and lignin in herbage. J. Sci. Food Agric. 12, 152–160.

Dewar, W.A., McDonald, P., Whittenbury, R., 1963. The hydrolysis of grass hemicelluloses during ensilage. J. Sci. Food Agric. 14, 411–417.

Duncan, D.B., 1955. Multiple range test and multiple. F. Test Biometrics 11, 1–42.

Galligan, W., Reese, E.T., 1954. Evidence of for multiple components in microbial cellulases. Can. J. Microbiol. 1, 90–107.

George, F.C., Melvin, T.Y., 1979. Analysis of fiber components in feed and forages using gas–liquid chromatography. J. Agric. Food Chem. 27, 373–377.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analysis Apparatus, Reagents, Procedures and Some Applications. Agriculture Handbook, Vol. 379. ARS-USDA, Washington, DC.

Mastsuoka, S., Branda, L.N., Fujita, H., 1997. Breakdown of structural carbohydrates during the ensiling process of grasses treated with *Lactobacillus* inoculant and cellulose preparation and subequent effects on their in vitro digestibility. Jpn. Anim. Sci. Technol. 68, 661–667.

McDonald, P., Whittenbury, R., 1977. The ensilage process. In: Butler, G.W., Bailey, R.W. (Eds.), Chemistry and Biochemistry of Herbage, Vol. 3. Academic Press, NY, pp. 33–36.

McDonald, P., Henderson, A.R., Heron, S.J.E., 1991. Principle of ensilage. In: McDonald, P., Henderson, A.R., Heron, S.J.E. (Eds.), The Biochemistry of Silage, 2rd Edition. Chalcombe Publications, Lincol, UK, pp. 9–40.

McDonald, P., Stirling, A.C., Henderson, A.R., Whittenbury, R., 1962. Fermentation studies in wet herbage. J. Sci. Food Agric. 13, 581–590.

McDonald, P., Sterling, A.C., Henderson, A.R., Dewar, W.A., Stark, G.H., Macpherson, H.T., Reid, A.M., Slater, J., 1960. Studies on ensilage. Edin. Sch. Agric. Tech. Bull. 24, 1–83.

Michael, K.W., 1984. In: Allen, I.L., Richard, I.M. (Eds.), The Silage Fermentation. Marcel Dekker, New York, pp. 1–22.

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.

Morrison, I.M., 1988. Influence of some chemical and biological additives on the fiber fraction of lucerme on ensilage in laboratory silo. J. Agric. Sci. 11, 35–39.

Taylor, K.A., Buchnan-Smith, 1992. A calorimetric method for the qua-ntitation of uronic acids and specific assay for galacturonic acid. Anal. Biochem. 201, 190–196.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods of dietary fiber, neutral detergent fiber and non starch poly-saccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Wilkins, R.J., 1981, The nutritive value of silage. In: Haresign, W., Cole, D.J.A (Eds.), Recent Developments in Ruminant Nutrition. pp. 140.

Zimmer, E. In: Proceedings of the British Grassland Society Occasional Symposium, Vol. 11. Brighton, pp. 189–197.