In Sacco Ruminal Degradation Characteristics of Chemical Components in Fresh Zoysia japonica and Miscanthus sinensis Growing in Japanese Native Pasture

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ABSTRACT: Ruminal degradation characteristics of dry matter (DM), neutral detergent fiber (NDF) and crude protein (CP) in fresh leaves of two Japanese native grasses (Zoysia japonica and Miscanthus sinensis) and one sown temperate grass (Dactylis glomerata) were investigated by an in sacco method in spring (mid-May), summer (mid-July) and autumn (mid-September). Japanese native grasses had higher NDF and lower CP concentrations than D. glomerata, and the CP concentration in native grasses decreased in autumn. Ruminal degradability of DM, NDF and CP was lower in native grasses than in D. glomerata (p<0.05) in all seasons. DM and NDF degradability decreased in summer for Z. japonica and D. glomerata, while it decreased in autumn for M. sinensis. CP degradability in Z. japonica was constant throughout the seasons, whereas that in M. sinensis greatly decreased in summer and autumn (p<0.05). It was concluded that Z. japonica could stably supply ruminally digestible nutrients for grazing animals in Japanese native pasture. However, the degradation characteristics of freshly chopped native grasses did not fit the exponential model of D=a(1-e^-ct) proposed by Orskov and McDonald. (Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 41-47)

Key Words: Dactylis glomerata, Fresh Material, In Sacco Degradation, Japanese Native Pasture, Miscanthus sinensis, Zoysia japonica

INTRODUCTION

Zoysia japonica and Miscanthus sinensis are major plant species having a wide distribution in native Eastern Asian pastures (Matsumura, 1996, 1997). The native pasture in Japan has been utilized for grazing forage for Japanese native beef cattle for more than a hundred and fifty years (Kato, 1987). Although the productivity of feed (Sato et al., 1975; Ogawa et al., 1996) and animal performance (Hayashi et al., 1965, 1967, 1979) in native pastures was lower than in sown pastures, these native grasses have enough nutritive value to maintain the body weight of nonlactating and nonpregnant beef cows (Sawamura et al., 1983; Kosako et al., 1999).

An understanding of the ruminal degradation characteristics of native forage species and their chemical composition is important to predict the intake and the digestibility of the native pasture for grazing animals. Forage species differing widely in ruminal digestibility may differ only slightly in chemical composition due to their physical properties (e.g., Smith et al., 1971; Bowman and Firkins, 1995). Ruminal dry matter (DM) degradability of Z. japonica and M. sinensis after 48 h of in sacco incubation decreased in summer and autumn, which was different with seasonal change in chemical composition (APPRC, 1987). Therefore, it is necessary to obtain information on ruminal degradation characteristics of some chemical components in Japanese native forage and also on their seasonal change.

Many studies have shown that nutritive value (Minson, 1966), digestibility (López et al., 1995; Vanbatalo and Varvikko, 1989) and passage rate of digesta (Pasha et al., 1994) of fresh forage were affected by freezing and drying. Recently, Ogura et al. (1999) proposed that fresh material might be desirable to evaluate nutritive value and digestibility of Japanese native grasses. However, there have been few studies focused on the ruminal degradation characteristics of fresh native grass.

The objective of this study was to estimate the ruminal degradability of chemical components in fresh Japanese native grasses by an in sacco method, and also their seasonal change.

MATERIALS AND METHODS

Three nonpregnant and nonlactating Japanese black beef cows (500±67 kg) with ruminal fistula (Fujihira Kogyo, Co. Ltd, Tokyo, i.d. 100 mm) were placed in a stanchion stall. The animals were fed on Italian ryegrass hay at 12.5 g/kg body weight twice a day (09:00 and 16:00), and had free access to water and commercial mineral salt.

Two native grass species, Z. japonica and M. sinensis, and sown temperate grass, Dactylis glomerata, were harvested from native grazing pastures at the
Chugoku National Agricultural Experiment Station, Shimane prefecture, Japan, in mid-spring (11 May), mid-summer (13 July), and mid-autumn (14 September) in 1998. *Z. japonica* and *M. sinensis* were sampled from a native pasture that was continuously grazed by Japanese black beef cows from 21 April to 7 December in 1998. *D. glomerata* was sampled from a native pasture that was rotationally grazed by 12 to 26 Japanese black beef cows 4 times in 1998. The two native pastures were adjacent to each other, and they had not been fertilized for several years.

Forage was sampled in the morning on days without rainfall. Cutting height was approximately 2, 5 and 20 cm for *Z. japonica*, *D. glomerata* and *M. sinensis*, respectively, above ground. The mean plant heights of sampled grass species are shown in table 1. Because the mean leaf length of *Z. japonica* was approximately 8 cm, green laminae of *M. sinensis* and *D. glomerata* were chopped into lengths of approximately 8 cm using a kitchen knife (table 1). Parts of the fresh samples were dried at 105°C for 16 h to determine DM concentration. The dried samples were ground into particles sufficiently small to pass through a 1 mm mesh, and the concentrations of neutral detergent fiber (NDF) (AOAC, 1988) and crude protein (CP) (AOAC, 1984) were measured.

Approximately 5 g quantities on a DM basis of the freshly chopped sample were placed in polyester nylon bags (20 cm x 10 cm, pore size 42±2 μm, EX screen, NBC Industries Co. Ltd, Tokyo). Bags were sealed by heating with a desk sealer. Sixteen bags of each forage species and 500 grams of anchor weight were placed in a large nylon mesh sack (42 cm x 42 cm, mesh size 3 mm x 5 mm). Bags were soaked in water (39°C) for 20 min before ruminal incubation to exclude all air in bags and to saturate forage samples with moisture. Bags were placed in the rumen just before feeding time. Each animal received a total of 48 bags. Two bags were removed from the rumen at intervals of 8, 16, 24, 36, 48, 72, 96 and 120 h for each grass species. After removal from the rumen, bags were rinsed gently in tap water until the rinse water ran clear, gently hand squeezed to remove excess water, and dried at 105°C for 16 h. Incubation residues were bulked by incubation time, ground (1-mm mesh) and analyzed for the disappearance rate of DM, NDF and CP.

The DM, NDF and CP degradability data were analyzed statistically using a split-plot ANOVA for two-way factorial with the GLM procedures of SAS (1988). Main plots were forage species and season harvested, and the subplot was incubation time. Differences among mean values of forage species within each season and within each incubation time were also compared using the least significant difference (LSD) test supported by a significant F-test.

The ruminal degradation characteristics of forage were estimated by fitting degradability data to the equation D=a+b(1-e^−ct), where D=disappeared DM, NDF and CP (g/g) after t hours estimated by the in sacco method, and a, b and c are constants representing the degradation parameters (Ottskov and McDonald, 1979). Calculations were done by using the non-linear technique (PROC NLIN) of SAS (SAS, 1988). Potentially digestible fraction (g/g) and rate of disappearance (%/h) were determined as a+b and c, respectively. The constant a was set to zero for estimating NDF degradation characteristics because, by definition, NDF is assumed insoluble (Beauchemin, 1992).

### RESULTS

The chemical compositions of the three grass species are shown in table 2. DM and NDF concentrations were higher in two native grasses than in *D. glomerata* for all seasons. In all grasses, however, DM concentration increased and NDF concentration decreased with the progress of seasons. CP concentrations were consistently high in *D. glomerata* (156 to 171 g/kg DM) throughout the seasons, while CP concentration in native grasses was

| Table 1. Plant length in the pasture and the length of chopped leaves in *Z. japonica*, *M. sinensis* and *D. glomerata* |
|---------------------------------|---------------------------------|---------------------------------|
| **Plant length in the pasture (cm)** | **May** | **July** | **September** |
| *Z. japonica* | 8.0 ± 0.30 | 8.1 ± 0.46 | 10.8 ± 0.77 |
| *M. sinensis* | 74.2 ± 3.62 | 86.0 ± 4.46 | 96.1 ± 10.19 |
| *D. glomerata* | 74.9 ± 3.03 | 52.8 ± 2.44 | 36.4 ± 2.09 |
| **Length of chopped leaves (cm)** | **May** | **July** | **September** |
| *Z. japonica* | 7.91 ± 0.39 | 7.51 ± 0.18 | 7.95 ± 0.02 |
| *M. sinensis* | 7.75 ± 0.11 | 8.07 ± 0.04 | 7.73 ± 0.40 |
| *D. glomerata* | 8.18 ± 0.27 | 7.92 ± 0.12 | 7.90 ± 0.08 |

* Mean ± standard error (n=10).

* Mean ± standard error (n=30).
Table 2. Dry matter (DM), neutral detergent fiber (NDF) and crude protein (CP) concentrations in Z. japonica, M. sinensis and D. glomerata

|               | May   | July  | September |
|---------------|-------|-------|-----------|
| Dry matter (g/kg fresh matter) |       |       |           |
| Z. japonica   | 275   | 389   | 426       |
| M. sinensis   | 223   | 295   | 341       |
| D. glomerata  | 196   | 226   | 280       |
| NDF (g/kg DM) |       |       |           |
| Z. japonica   | 829   | 802   | 791       |
| M. sinensis   | 787   | 777   | 766       |
| D. glomerata  | 696   | 659   | 650       |
| CP (g/kg DM)  |       |       |           |
| Z. japonica   | 84    | 87    | 69        |
| M. sinensis   | 94    | 102   | 71        |
| D. glomerata  | 166   | 156   | 171       |

The degradabilities of native grasses were expressed as the mean of three replications ± standard error. The degradabilities are expressed on an NDF basis. NDF degradability was calculated as a percentage of the degradation of NDF at each incubation time. DM degradability was calculated as a percentage of the degradation of DM at each incubation time.

The degradability of Z. japonica was higher than that of M. sinensis and D. glomerata in all seasons. In general, the degradability of Z. japonica was higher than that of M. sinensis and D. glomerata in all seasons.

However, it increased up to the level of spring by 120 h incubation. The degradabilities of native grasses in spring, summer and autumn were 0.549, 0.412 and 0.492 g/g for Z. japonica, and 0.618, 0.540 and 0.434 g/g for M. sinensis, respectively, after 120 h incubation.

NDF degradability of native grasses was also lower than that of D. glomerata (p<0.001) (figure 2). However, Z. japonica had higher mean NDF degradability than M. sinensis (p<0.05). In spring and summer, NDF degradability of M. sinensis was lower than that of Z. japonica at 120 h incubation. The degradability of D. glomerata in summer was lower than in other seasons (p<0.05), and it did not level off with 120 h incubation.

CP degradabilities of native grasses were lower than that of D. glomerata (p<0.001), and their seasonal change differed with species (figure 3). The degradability of Z. japonica showed similar values at the same incubation time throughout the seasons (p>0.1), and it was degraded more than 0.6 g/g with 120 h incubation. The degradability of M. sinensis was comparable to that of Z. japonica in spring; however, it decreased significantly in summer and autumn (p<0.05). In summer and autumn, the degradability of M. sinensis stagnated with 24 h to 48 h incubation (0.216-0.244 g/g for summer and 0.188-0.211 g/g for autumn, respectively). Although CP degradability of M. sinensis in summer increased after 72 h and reached the same level in spring (0.670 and 0.641 g/g with 120 h incubation for spring and summer, respectively), it fell to 0.392 g/g with 120 h incubation in autumn.

Figure 1. Dry matter (DM) disappearance in fresh Z. japonica, M. sinensis and D. glomerata leaves harvested in spring (mid-May) (−−−−), summer (mid-July) (−−−−) and autumn (mid-September) (−−−−). Values are the means (n=3) with their standard errors represented by vertical bars.
On the other hand, CP degradability of *D. glomerata* reached 0.943-0.966 g/g after 72 h incubation, and CP disappeared almost completely with 120 h incubation (0.981 to 0.991 g/g). Potentially digestible fraction and the rate of disappearance of forage estimated by non-linear regression are shown in table 3. The degradability of native grasses did not fit the exponential model in some cases, and parameters could not be determined (denoted ND in table 3). For *M. sinensis*, parameters could not be obtained, except for the degradability of DM in spring and autumn, and of CP in spring. Potentially digestible fraction of *D. glomerata* exceeded 1.0 g/g for the degradability of NDF and CP in summer and CP in autumn. Rate of disappearance in *D. glomerata* was high in all seasons and components.

**Figure 2.** Neutral detergent fiber (NDF) disappearance in fresh *Z. japonica*, *M. sinensis* and *D. glomerata* leaves harvested in spring (mid-May) (—△—), summer (mid-July) (—●—) and autumn (mid-September) (—□—). Values are the means (n=3) with their standard errors represented by vertical bars.

**Figure 3.** Crude protein (CP) disappearance in fresh *Z. japonica*, *M. sinensis* and *D. glomerata* leaves harvested in spring (mid-May) (—△—), summer (mid-July) (—●—) and autumn (mid-September) (—□—). Values are the means (n=3) with their standard errors represented by vertical bars.
Table 3. Ruminal degradation characteristics of dry matter (DM), neutral detergent fiber (NDF) and crude protein (CP) in fresh *Zoysia japonica*, *Miscanthus sinensis* and *Dactylis glomerata* estimated by the model $D = a + b(1 - e^{-t})$ proposed by Ørskov and McDonald (1979)

|                | *Z. japonica* |       |       |       | *M. sinensis* |       |       |       | *D. glomerata* |       |       |
|----------------|---------------|-------|-------|-------|---------------|-------|-------|-------|----------------|-------|-------|
|                | May           | July  | Sept. | May   | July          | Sept. | May   | July  | Sept.          | May   | July  |
| DM;            |               |       |       |       |               |       |       |       |                 |       |       |
| Potentially digestible fraction (a+b, g/g) | 0.849 | 0.664 | 0.782 | 0.780 | ND            | 0.864 | 0.839 | 0.815 | 0.849          |       |       |
| Rate of disappearance (c, %/h) | 0.83 | 0.83  | 0.74  | 1.26  | ND            | 0.64  | 3.68  | 2.79  | 3.32           |       |       |
| Residual standard deviation$^a$ | 0.0296 | 0.0226 | 0.0304 | 0.0557 | ND            | 0.0309 | 0.0240 | 0.0550 | 0.0512         |       |       |
| NDF;           |               |       |       |       |               |       |       |       |                 |       |       |
| Potentially digestible fraction (a+b, g/g) | 0.783 | ND$^b$ | 0.923 | ND    | ND            | ND    | 0.852 | 1.107 | 0.916          |       |       |
| Rate of disappearance (c, %/h) | 1.00 | ND   | 0.55  | ND    | ND            | ND    | 2.67  | 0.96  | 1.78           |       |       |
| Residual standard deviation$^a$ | 0.0361 | ND   | 0.0363 | ND    | ND            | ND    | 0.0407 | 0.0935 | 0.0794         |       |       |
| CP;            |               |       |       |       |               |       |       |       |                 |       |       |
| Potentially digestible fraction (a+b, g/g) | 0.661 | 0.699 | 0.596 | 0.857 | ND            | ND    | 0.992 | 1.010 | 1.008          |       |       |
| Rate of disappearance (c, %/h) | 2.51 | 2.05  | 3.33  | 1.16  | ND            | ND    | 5.50  | 3.81  | 4.49           |       |       |
| Residual standard deviation$^a$ | 0.0348 | 0.0473 | 0.0500 | 0.0536 | ND            | ND    | 0.0385 | 0.0740 | 0.0503         |       |       |

$^a$ n=24.

$^b$ No data (unrecorded).

compared with native grasses; the rate of disappearance for CP in spring was 5.50 %/h in *D. glomerata*, which was greater than the 2.51 %/h of *Z. japonica* and the 1.16 %/h of *M. sinensis*. The degradabilities of *Z. japonica* showed a relatively low residual standard deviation compared with the other grasses.

**DISCUSSION**

The present study showed that although the chemical composition of *Z. japonica* and *M. sinensis* changed similarly, their ruminal degradation characteristics greatly differed with season. *Z. japonica* offered in this experiment bloomed in mid-May, when its NDF concentration and ruminal degradability were highest, whereas *M. sinensis* had not yet bloomed in mid-September when ruminal degradability was remarkably reduced. It is known that Japanese tropical native grasses have high lignification compared with temperate grasses (Sakurai, 1963), particularly in summer and autumn, and that *M. sinensis* has a higher lignin concentration than *Z. japonica* (AFFRC, 1987) which has nearly the same lignin concentration as Italian ryegrass (Ozawa et al., 1995). The reduction of ruminal degradability in *M. sinensis* in autumn is suggested to be due to increased coarseness caused by the change of structural characteristics of leaf tissue with lignification. In contrast, for *Z. japonica*, it has also been suggested that the seasonal change in leaf tissues is relatively small. It has also been reported that leaf protein in temperate grass is widely and abundantly distributed in the leaf blade, whereas in tropical grasses, such as *Z. japonica* and *M. sinensis*, it was mainly concentrated in the leaf bundle sheath (Sakurai, 1963). In general, bundle sheaths of warm-season grasses are digested less rapidly than those of cool-season grasses, but differences are also found even within cultivars of a species (Akin, 1979). These findings indicate that seasonal change in morphological and digestive characteristics could differ in *Z. japonica* and *M. sinensis*, even though their seasonal change in chemical composition is similar. The decline in ruminal CP degradability of *M. sinensis* in autumn may be due to the increased concentration of ruminally undegradable protein with increasing lignification, which is not found in *Z. japonica*.

Potential ruminal degradability in freshly chopped native forage could not be determined (table 3). Forage species have a cuticle which is resistant to microbial access and attack of epidermal and underlying tissues (Pond et al., 1984). This barrier for ruminal digestion is more resistant in tropical grass, but it could be dislodged by mastication of animal (Pond et al., 1984). In the present study, because the forage used had been freshly cut but not masticated, plant barriers such as the cuticle were not disrupted and probably inhibited ruminal microbial digestion. The distinctive change in degradability for *M. sinensis* in summer and autumn (i.e. the stagnation of degradability with 24 to 48 h incubation) also might be partly due to this reason, and partly due to the greater heterogeneity of plant tissues (López et al., 1995) having different degradabilities (Akin and Burdick, 1975; Akin, 1979; Akin et al., 1983; Wilson et al., 1983) compared with other grasses. As a consequence, rates of digestion of native grasses might be low, and degradability might not level off with 120
h incubation. Unreasonable parameter values were also obtained for *D. glomerata*; that is, the potential degradable fraction exceeded 1.0 g/g for NDF in summer, and for CP in summer and autumn (table 3). López et al. (1995) estimated the potential DM degradability and rate of degradation of freshly chopped ryegrass as 0.872 g/g and 0.048 %/h, respectively, by an *in situ* technique. However, the length of fresh samples used in their experiment was from 5 to 15 mm, which was shorter than that in the present study (8 cm). Therefore, relatively low rates of digestion of long, fresh *D. glomerata* might make the parameter of potentially degradable fraction greater than 1.0 g/g by delaying the plateau level. This indicates that long, fresh forage has a lower rate of digestion, even though the forage is a highly digestible, temperate species.

Despite the low nutritive value and ruminal degradability of Japanese native forage, animal diet quality may be improvable. Mastication by the animal increased the *in situ* ruminal degradability of the forage (Beauchemin, 1992; López et al., 1995). In Japanese native grasses, the increased dry matter degradability in a masticated sample was also observed *in vitro* (Ogura et al., 1999). Furthermore, it has been reported that grazing animals changed their diet by selective grazing with seasonal variation of vegetation in a young tree plantation (Hasegawa et al., 1999). These findings indicate that evaluation of the digestion characteristics of ruminant ingesta (*i.e.*, extrusa sampled via esophageal fistula) is important to estimate the efficiency of nutrient utilization by animals in Japanese native pasture.

**CONCLUSION**

The species *Z. japonica* and *M. sinensis*, which are widely distributed in native pasture in Japan and fed to ruminants, have poor nutrient and ruminal degradability compared with *D. glomerata*. However, in *Z. japonica*, both chemical composition and ruminal CP degradability were relatively stable throughout the season, whereas ruminal degradability in *M. sinensis* decreased remarkably in autumn. It was concluded that *Z. japonica* could stably supply ruminally digestible nutrients for grazing animals throughout the grazing seasons. Further study is needed to evaluate the effects of mastication of animals on the kinetics of ruminal degradation of diet, as well as of foraging behavior.

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