THE EFFECT OF MATURITY OF ITALIAN RYEGRASS (*Lolium multiflorum*, L) ON *IN VITRO* RUMEN DIGESTION AND GAS PRODUCTION

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Summary

Three stages of growth of Italian ryegrass (pre-blooming, P-B; early-blooming, E-B; and late-blooming, L-B) were used to evaluate the effect of maturity on *in vitro* digestion of dry matter, fiber components and gas production. The rumen digestibility and gas production values were obtained by incubation of each sample in the rumen fluid of sheep for 12, 24, 36, 48 and 72 hr, respectively. The results showed that digestibility of dry matter (DM) significantly reduced ($p < 0.05$) as advancing maturity of the grass. Similarly, the digestibility of neutral detergent fiber (NDF) and acid detergent fiber (ADF) also significantly decreased ($p < 0.05$) with advancing maturity at all incubation times. However, the effect of maturity on digestibility of cellulose and hemicellulose was only detected when the samples were incubated more than 36 hr, where L-B was lower than P-B and E-B. Potential digestibility of nutrients, the maximum digestibility attainable in the rumen theoretically, was also higher at P-B than those of E-B and L-B. The amount of gas produced by microbial fermentation was closely related to the extent of DM digestion, and it was negatively correlated with advancing maturity of the grass.

(**Key Words** : Italian Ryegrass, Maturity, *In vitro* Digestion, Gas Production)

Introduction

It has been recognized for a long time that the stage of growth or maturity is one of the major factors that directly affects the quality of forage. Evidence from several experiments indicates that changes in maturity are accompained by increases in proportion of fibrous components and decreases in soluble constituents, thus resulting in a low nutrient digestibility and low voluntary intake of the forage (Sulivan, 1973; Hume and Purser, 1974; Ventura et al., 1975; Cleale and Bull, 1986 and Balde et al., 1993). The depression in rumen digestibility of nutrients is closely related with a higher lignin content in a more mature grass (Jones and Wilson, 1987; Minson, 1990 and Flachowsky et al., 1993). Furthermore, Jones and Wilson (1987) suggested that the association of polysaccharides of cell wall with lignin hinders attack by microbial enzymes and prevents the physical attachment of bacteria to the cell walls.

Several researchers have also reported that the amount of gas released when a feed is incubated *in vitro* in rumen fluid, is closely related to rumen digestibility of the feed (Trei et al., 1970, Menke et al., 1979 and Khazaal et al., 1994) and could be used to predict the rumen digestibility and feed intake. In a previous study (Fariani et al., 1994), we reported that the nutritive value of Italian ryegrass, based on its chemical properties and *in sacco* rumen digestibility, were obviously reduced with advancing maturity. Objectives of the present experiment were to investigate the effect of maturity on *in vitro* rumen digestibility of dry matter, fiber components of Italian ryegrass, gas production and their relationships.

Materials and Methods

Experimental grass

Italian ryegrass was seeded at the end of October 1991 at the farm of the Faculty of Agriculture, Shimane University, Japan. Representative samples were obtained from the grass harvested at three different growth stages, i.e : pre-blooming, P-B (April, 8, 1992), early-blooming, E-B (May 4, 1992) and late-blooming, L-B (May 30, 1992). The stages of growth at harvest were described according to stage of maturity terms used in the international feed names reported by Harris (1970). Thus

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pre-blooming is the stage when stem begins elongation until just before blooming. Early-blooming is the stage between initiation of bloom and stage at which one-tenth of the plants are in bloom, and late-blooming is the stage at which blossoms begin to dry and fall and seeds begin to form.

Immediately after harvesting, the samples were dried in a forced air oven at 60℃ for 24 hr and ground through a 1 mm screen. Chemical composition of the grass was analyzed by the standard method of the Association of Official Analytical Chemists (AOAC, 1984), and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined according to the procedures of Goering and Van Soest (1970).

Procedures for determination of *in vitro* digestion and measurement of gas production

Two Japanese corriedale wethers fitted with rumen fistula (45.4 ± 2.1 kg of B.W.) were used as source of rumen fluid. The sheep were fed a daily diet of hay (2% of B.W), 200 g concentrate (60% wheat bran and 40% barley) and 1 g vitamin premix to meet their maintenance requirements. A mineralized salt block and water were freely available. The daily allowance of hay and concentrate were given in two equal portions twice daily at 09:00 and 17:00 hr, and they had been maintained on this diet for 2 weeks before starting, and also throughout the experimental period. Rumen fluid was withdrawn via fistula just before the morning feed and then strained through four layers of cheesecloth. One part of the rumen fluid was mixed with two parts of the medium consisting of *in vitro* rumen buffer solution, macro and micro mineral solutions, resazurine and reduction solutions as described by Goering and Van Soest (1970). One gram of each sample was incubated in the rumen fluid medium-mixture for 12, 24, 36, 48 and 72 hr (stage 1). At the end of each incubation time, 1 ml toluene was added to each flask as a preservative and placed in a refrigerator. All of fermented residues at stage 1 were extracted by neutral and acid detergent solutions, respectively, for determination of NDF and ADF contents.

Gas production during *in vitro* microbial fermentation was measured by syringes according to the method of Menke et al. (1979). Readings were taken at 12, 24, 36, 48 and 72 hr after incubation. Both measurements of digestibility and gas production of each sample were done in triplicates. The exponential equation described by Ørskov and Mc Donald (1979) was used in this study to determine the characteristics of *in vitro* rumen degradability as : $p = a + b(1 - e^{-ct})$, where,

p = rumen degradation at the time t,

a = intercept, which is highly correlated with water soluble fraction,

b = the portion of feed which is degraded at the time t,

c = the degradation rate of 'b' fraction, and

t = incubation time.

In addition, a + b (asymptote) shows the value of potential degradability in the rumen. This value is theoretically the maximum digestibility of nutrients attainable in the rumen.

Data from gas production were fitted to the exponential equation as described by Osuji et al. (1993) :

$Y = b(1 - e^{-ct})$, where,

Y = the volume of gas produced with time (t),

b = the potential extent of gas production.

c = the rate of gas production.

The intercept (a) is not included in the model with the understanding that no gas is produced from unfermented feed.

Statistical analysis

Data of digestion values and gas production were analyzed by a one-way analysis of variance. The model was :

$Y_{ij} = \mu + t_i + e_{ij}$, where,

Y_{ij} = the j_{th} observation in the i_{th} growth stage of grass,

μ = overall mean,

t_i = effect of the i_{th} growth stage of grass, and

e_{ij} = the error term.

The least significance differences test (LSD) was used to determine differences between the treatment means (Steel and Torrie, 1980). The constants of *in vitro* digestion and gas production characteristics were determined by analysis of non-linear regression (Gauss-Newton method) with the procedures of SAS (1985).

Results and Discussion

Chemical composition of Italian ryegrass used in the present study was the same as reported earlier (Fariani et al., 1994) in which, crude protein obviously reduced from 17.1% at P-B to 9.4 and 7.8% at E-B and L-B, respectively. The fiber components increased as advancing maturity of the grass. Concentration of NDF ranged from 48.3% to 64.4%, while concentration of ADF increased from 30.0% at P-B to 46.4% at L-B. Lignin concentration increased more than threefold over the 3 harvesting stages (1.3 vs 5%). According to Sulivan (1973), nutritive value of a forage depends upon the morphological and physiological changes which take place during each stage of growth. As the forage matures, the cytoplasmic portion

of each cell becomes less important and the quantity of some of its constituents such as protein, lipids, soluble carbohydrates and soluble minerals decreases. However, the cell walls become relatively more important, and the fibrous constituents increase and become more lignified. These findings are also consistent with results of Messman et al. (1991) for bromegrass, Bowman et al. (1991) for orchard grass, Nelson and Satter (1992) for alfalfa and Cheney et al. (1993) for perennial grasses, in which cases protein concentration decreased while fiber fractions increase rapidly with advancing maturity.

In vitro DM digestibility is presented in figure 1. The DM digestibility was significantly affected by maturity ($p < 0.05$) at all incubation times. At 72 hr incubation time, for instance, the DM digestibility averaged 87.50, 75.36 and 55.28% for P-B, E-B and L-B, respectively. This indicated that the indigestible fraction increased with increasing maturity. Results obtained from fitting these data to the exponential equation shows that potential digestibility (a + b) of DM markedly reduced from 89.87% at P-B to 60.20% at L-B. Similarly, rate of DM digestion decreased from 5.5 %/hr at PB to 2.9 %/hr at LB (table 1). These findings suggest that the higher concentrations of fiber (mainly NDF, ADF and lignin) in the more mature grass, has prevented plant materials from rumen microbial attack, thus resulting in a lower rate and extent of digestion. Similar results have also been obtained in the earlier study on *in sacco* digestion trials (Fariani et al., 1994), that the rate, extent and potential degradability of DM of Italian ryegrass decreased with increasing maturity.

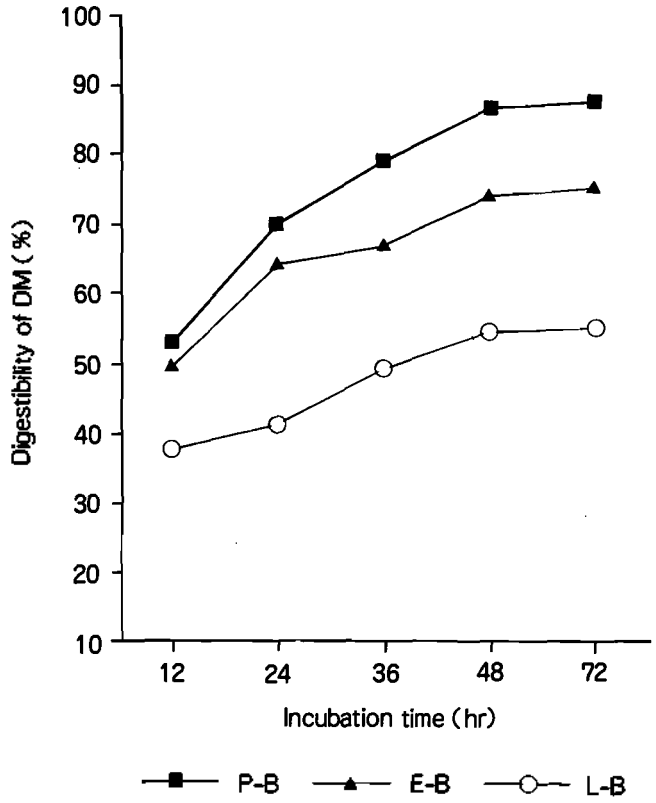

Figure 1. *In vitro* digestibility of dry matter

The effect of harvesting stages on *in vitro* digestion of NDF is presented in figure 2. It is clear that the extent of NDF digestion was significantly higher ($p < 0.05$)at P-B than that of E-B and L-B. The potential degradability of NDF was decreased from 83.88% at P-B to 66.76 and 59.79% at E-B and L-B, respectively. Rate of NDF digestion was relatively lower at L-B (4.1 %/hr), but it was not different among the P-B and E-B (table 2). This may be associated with increasing fiber content, particularly lignin, of the grass. The amount of lignin as a percentage of NDF increased with maturity from 2.63% at P-B to 5.59 and 7.76% at E-B and L-B, respectively. Thus, the higher lignin content will produce a much stronger fiber-lignin bonds, which prevents the swelling of fiber to a state suitable for penetration by rumen microorganism.

These findings are in agreement with the results of Brown (1988), that the extent of *in vitro* NDF disappearance of stargrass reduced from 66% to 49.5% when the harvesting time was delayed from 5 to 10 weeks re growth. The rate of NDF disappearance was also reduced from 5.8 %/hr at 5 weeks re growth to 4.0 %/hr

TABLE 1. DIGESTION CHARACTERISTICS OF DRY MATTER ACCORDING TO THE EQUATION $P = A + B (1 - E^{-ct})$

Harvesting stages	a*	b**	(a + b)	c#	Residual s.d
		(%)			
Pre blooming	18.18	71.69	89.87	0.055	1.21
Early blooming	24.47	51.92	76.39	0.055	2.11
Late blooming	26.71	33.89	60.20	0.029	2.49

*a = intercept (water soluble fraction).
**b = the portion of dry matter which is degraded at the time t.
*c = the degradation rate of 'b' fraction.

at 10 weeks of re growth.

As can be seen in figure 3, advancing maturity of the grass was associated with significant decrease ($p < 0.05$) in the extent of *in vitro* ADF digestion at all the

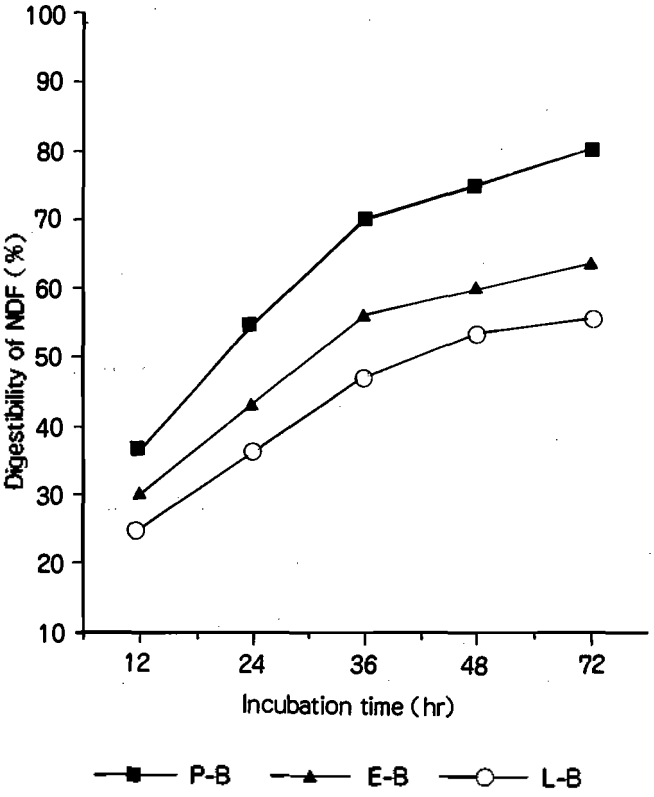

Figure 2. *In vitro* digestibility of NDF

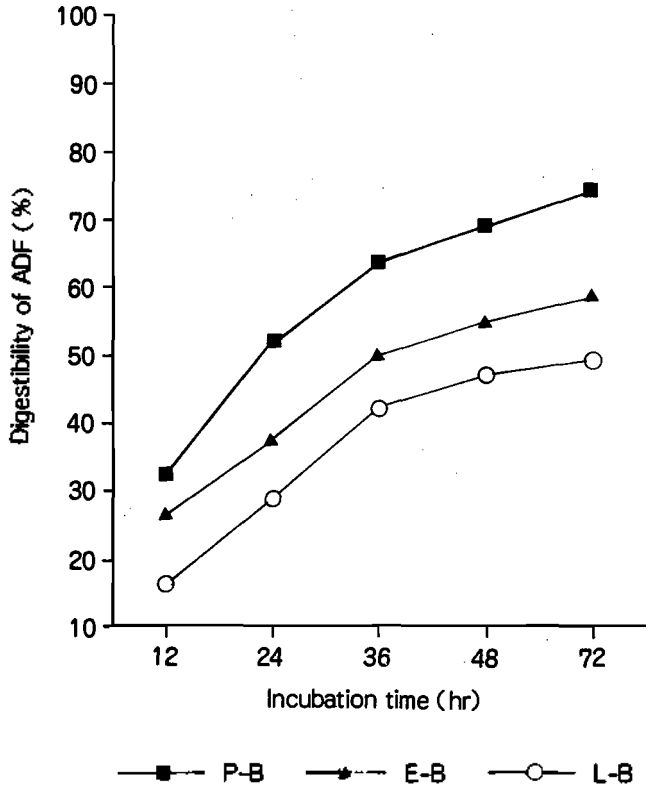

Figure 3. *In vitro* digestibility of ADF

TABLE 2. DIGESTION CHARACTERISTICS OF NDF ACCORDING TO THE EQUATION $P = A + B(1 - E^{-ct})$

Harvesting stages	a*	b**	(a + b)	c#	Residual s.d
		(%)			
Pre blooming	0.36	83.2	83.88	0.046	0.04
Early blooming	2.39	64.37	66.76	0.045	1.64
Late blooming	1.66	58.13	59.79	0.041	1.70

*a = intercept (water soluble fraction).
**b = the portion of NDF which is degraded at the time t.
#c = the degradation rate of 'b' fraction.

incubation times. The potential digestibility of ADF was greatly reduced by increasing maturity of the grass. The values were 76.46, 62.86 and 52.51% for P-B, E-B and L-B, respectively. It is not clear, however, why the rate of ADF digestion was not different between P-B and L-B (5 vs 4.7 %/hr), with the lowest value occurring at E-B (4 %/hr) (table 3). This may also directly reflect the increasing proportion of lignin to ADF as maturity of the grass increases. The amount of lignin as percentage of ADF was 4.33, 7.63 and 10.78% at P-B, E-B and L-B, respectively. A study of Bowman et al. (1991) with orchard grass showed that the extents of NDF and ADF digestion were obviously affected by the forage maturity. They obtained that the digestibility of NDF and ADF at 96 hr incubation time averaged 67% and were greater at early maturity than later. The results obtained in the present study were also very consistent with the results reported earlier for *in sacco* trials (Fariani et al., 1994), and strongly support the results of Chemey et al. (1993) that *in vitro* accumulation of indigestible fiber residue increased with harvest dates of perennial grasses.

In agreement with the previous study on *in sacco*

TABLE 3. DIGESTION CHARACTERISTICS OF ADF ACCORDING TO THE EQUATION $P = A + B(1 - E^{-\alpha})$

Harvesting stages	a*	b**	(a + b)	c#	Residual s.d
		(%)			
Pre blooming	−3.86	80.32	76.46	0.050	0.04
Early blooming	3.53	59.33	62.86	0.040	2.26
Late blooming	−12.51	65.02	52.51	0.047	2.66

*a = intercept (water soluble fraction).
**b = the portion of ADF which is degraded at the time t.
#c = the degradation rate of 'b' fraction.

digestion (Fariani et al., 1994), the extent of *in vitro* cellulose digestion was significantly lower ($p < 0.05$) at E-B and L-B than at P-B, when the forage was incubated more than 36 hr. However, no significant differences were observed between E-B and L-B at all the incubation times (figure 4). Consistent with these results, the potential digestibility of cellulose was higher for P-B than those for E-B and L-B (table 4). Effect of harvesting stages on the extent of hemicellulose digestion is presented in figure 5, while the characteristics of its digestion is in table 5. As with other digestion kinetics parameters, the rate and extent of hemicellulose digestion were significantly reduced with advancing maturity of the grass. The pattern of *in vitro* hemicellulose digestion was the same as for cellulose, in which significant differences were only detected after 36 hrs of incubation time. The highest values were found at P-B, followed by E-B and L-B, respectively. The reduction of cellulose and hemicellulose digestion due to increasing maturity was probably caused by higher contents of lignin and silica in the more mature grass. Minson (1990) reported that cellulose and hemicellulose can be divided into potentially digestible and indigestible fractions separated by a protective layer of lignin, in which the indigestible fractions will increase proportionally as advancing maturity of forage. Furthermore, Jones and Wilson (1987) pointed out that the polysaccharides of the cell wall of herbage are totally digestible when lignin is removed. Their association with lignin, however, hinders attack by microbial enzymes to a varying extent, depending primarily on the degree of lignification.

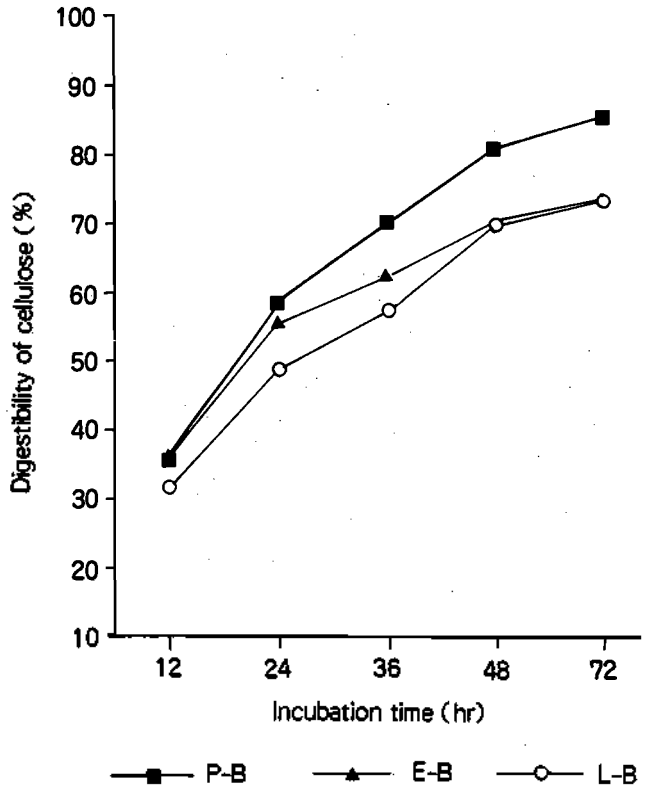

Figure 4. *In vitro* digestibility of cellulose

TABLE 4. DIGESTION CHARACTERISTICS OF CELLULOSE ACCORDING TO THE EQUATION $P = A + B(1 - E^{-\alpha})$

Harvesting stages	a*	b**	(a + b)	c#	Residual s.d
		(%)			
Pre blooming	−3.19	93.14	89.95	0.045	0.95
Early blooming	1.64	73.69	75.33	0.053	1.24
Late blooming	4.21	75.30	79.51	0.037	3.57

*a = intercept (water soluble fraction).
**b = the portion of cellulose which is degraded at the time t.
#c = the degradation rate of 'b' fraction.

Figure 6 shows the effect of harvesting stages on cumulative gas production during *in vitro* digestion trials. It can be seen that the pattern of gas production was very similar with the pattern of dry matter digestion, which

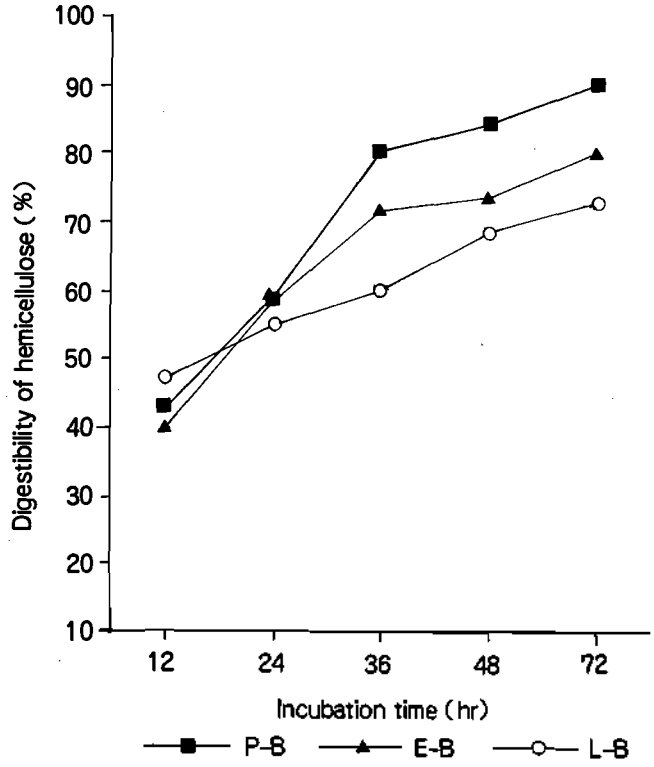

Figure 5. *In vitro* digestibility of hemicellulose

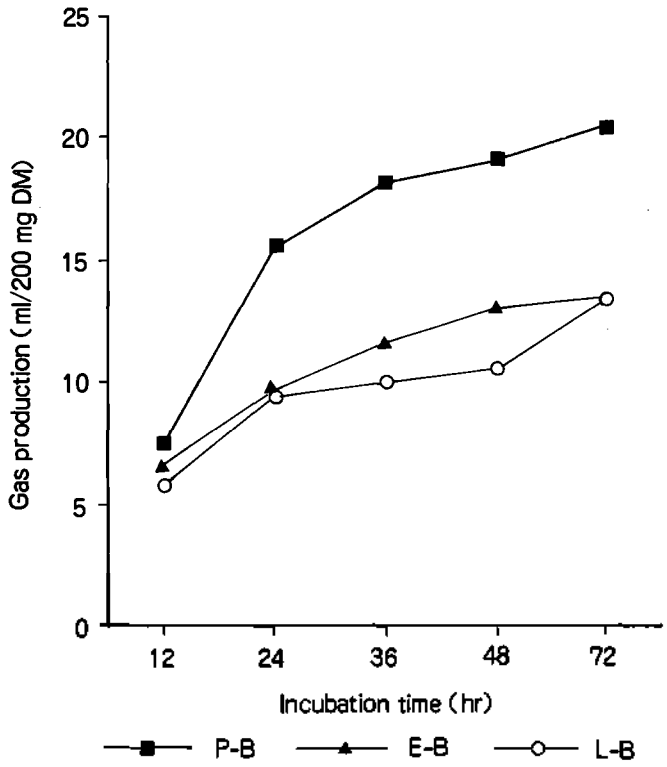

Figure 6. Cummulative *in vitro* gas production

TABLE 5. DIGESTION CHARACTERISTICS OF HEMICELLULOSE ACCORDING TO THE EQUATION $P = A + B (1 - E^{-ct})$

Harvesting stages	a*	b**	(a + b)	c#	Residual s.d
		(%)			
Pre blooming	5.60	89.67	85.27	0.043	1.21
Early blooming	3.92	77.48	81.40	0.052	1.68
Late blooming	36.66	47.23	83.89	0.021	1.48

*a = intercept (water soluble fraction).
**b = the portion of hemicellulose which is degraded at the time t.
*c = the degradation rate of 'b' fraction.

directly reflected the rate and extent of rumen microbial fermentation. With the exception of 12 hr incubation time, the gas production was significantly higher ($p < 0.05$) at P-B than at E-B and L-B. However, there were no significant differences between E-B and L-B at all incubation times. The amount of gas produced was closely related in a linear regression with *in vitro* dry matter digestibility, where the correlation coefficient (r) was 0.98, 0.99 and 0.88 for P-B, E-B and L-B, respectively. Data on the characteristics of gas production show that potential extent of gas production reduced from 21.76 ml/200 mg DM at P-B to 13.92 and 13.03 ml/200 mg DM at E-B and L-B, respectively (table 6). Khazaal et al. (1993) showed that the gas production from *in vitro* fermentation of various grasses (Lucerna, Sweet clover and Persian clover hay) harvested at different growth stages increased as increasing incubation time, but decreased as their maturity advanced. Furthermore, they reported that the gas productions were highly correlated with both *in sacco* and *in vivo* DM digestion. A similar finding was also reported by Menke et al. (1979).

In conclusion, the results reported in the present study

TABLE 6. CHARACTERISTICS OF GAS PRODUCTION ACCORDING TO THE EQUATION $Y = B(1 - E^{-\alpha})$

Harvesting stages	b* / 200 mg DM	c#	Residual s.d
Pre blooming	21.76	0.045	1.61
Early blooming	13.92	0.051	0.05
Late blooming	13.03	0.046	0.08

*b = the potential extent of gas production.
#c = the rate of gas production.

show that quality of Italian ryegrass, based on the *in vitro* digestibility, rapidly declined as advancing maturity. The gas production of *in vitro* fermentation was reduced with advancing maturity. It also could be concluded that although there is a little variation, *in vitro* digestion values of dry matter and fiber components, they were very comparable with the values of *in sacco* digestion trials reported earlier.

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Literature Cited

A.O.A.C. 1984. Official Methods of Analysis (13th Ed.). Association of Official Analitycal Chemists. Washington. D.C.

Balde, A. T., J. H. Vandersall, R. A. Erdman, J. B. Reeves and B. P. Glenn. 1993. Effect of Stage of Maturity of Alfalfa and Orchardgrass on *In Situ* Dry Matter and Crude Protein Degradability and Amino Acid Composition. Anim. Feed Sci. and Tech., 44:29-43.

Bowman, J. G. P., C. W. Hunt, M. S. Kerley and J. A. Paterson. 1991. Effects of Grass Maturity and Legume Substitution on Large Particle size Reduction and Small Particle Flow from the Rumen of Cattle. J. Anim. Sci. 69:369-378.

Brown, W. P. 1988. Maturity and Ammoniation Effects on the Feeding Value of Tropical Grass Hay. J. Anim. Sci., 66:2224-2232.

Cherney, D. J. R., J. H. Cherney and R. F. Lucey. 1993. *In vitro* Digestion Kinetics and Quality of Perennial Grasses as Influenced by Forage Maturity. J. Dairy. Sci. 76:790-797.

Cleale, R. M., IV and L. S. Bull. 1986. Effect of Forage Maturity on Ration Digestibility and Production by Dairy Cows. J. Dairy. Sci. 69:1587-1594.

Fariani, A., L. Warly, T. Matsui, T. Fujihara and T. Harumoto. 1994. Rumen Degradability of Italian Ryegrass (*Lolium multiflorum*, L) Harvested at Three Different Growth Stages in Sheep. Asian-Australasian. J. Anim. Sci. Vol. 7 (No. 1):41-48.

Flachowsky, G., W. Peyker, A. Schneider and K. Henkel. 1993. Fibre Analyses and *In Sacco* Degradability of Plant Fractions of Two Corn Varieties Harvested at Various Times. Anim. Feed Sci. and Tech. 43:41-50.

Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis. (apparatus, reagents, procedures and some application). Agric. Handbook 379, ARS, USDA, Washington, D.C.

Harris, L. E. 1970. Nutrition Research Techniques for Domestic and Wild Animals. Vol. 1. An International Record System and Procedures for analyzing Samples. p. 651.

Hume, I. D. and D. B. Purser. 1974. Aust. J. Agric. Res., 26:199-208.

Jones, D. I. H. and A. D. Wilson. 1987. Nutritive Quality of Forage. In: The Nutrition of Herbivores. (Ed. by: J. B. Hacker and J. H. Ternouth). Academic Press. pp. 65-89.

Khaazal, K., M. T. Dentinho, J. M. Riberio and E. R. Ørskov. 1993. A Comparison of Gas Production During Incubation With Rumen Contents *In Vitro* and Nylon Bag Degradability as Predictors of the Apparent Digestibility *In Vivo* and the Voluntary Intake of Hays. Brit. Soc. Anim. Prod. 57:105-112.

Khazaal, K., J. Boza and E. R. Ørskov. 1994. Assesment of Phenolics-Related Antinutritive Effects in Mediterranean Browse: A Comparison Between the Use of the *In Vitro* Gas Production Technique With or Without Insoluble Polyvinylpolypyrrolidone or Nylon Bag. Anim. Feed Sci. and Tech. 49:133-149.

Menke, K. H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider. 1979. The Estimation of the Digestibility and Metabolizable Energy Content of Ruminant Feedingstuffs From the Gas Production When They Are Incubated With Rumen Liquor *in vitro*. J. Agric. Sci. Camb. 93:217-222.

Messman, M. A., W. P. Weiss and D. O. Erickson. 1991. Effects of Nitrogen Fertilization and Maturity of Bromegrass on *in situ* Ruminal Digestion Kinetics of Fiber. J. Anim. Sci. 69:1151-1161.

Minson, D. J. 1990. Digestible Energy of Forage. In: Forage in Ruminant Nutrition. Academic Press. Inc. pp. 85-149.

Nelson, F. W. and L. D. Satter. 1992. Impact of Stage of Maturity and Method of Preservation of Alfalfa on

Digestion in Lactating Dairy Cows. J. Dairy. Sci. 75:1571-1580.

Ørskov, E. R. and Mc Donald, I. 1979. The Estimation of Protein degradability in the Rumen from Incubation Measurements Weighted According to Rate of Passage. J. Agric. Sci. Cambridge 92:499-503.

Osuji, P. O., I. V. Nsahlai and H. Khalili. 1993. Feed Evaluation. International Livestock Centre for Africa. Addis Ababa, Ethiopia.

SAS. 1985. SAS User's Guide. Basics. SAS., Inst., Inc., Carry, NC. Steel, R. G. D. and J. H. Torrie. 1981. Principles and Procedures of Statistics. A biometrical Approach (2nd Ed). Mcgraw-Hill International Book Company.

Sullivan, J. T. 1973. Drying and Storing Herbage as Hay. In: Chemistry and Biochemistry of Herbage. (Edited by Butler G. W. and R. W. Bailey). Academic Press. London and New York. pp. 1-31.

Trei, J., W. H. Hale and Brent Theurer, 1970. Effect of Grain Processing on *In Vitro* Gas Production. J. Anim. Sci. Vol. 30. 5:825-831.

Ventura, M., J. E. Moore, O. C. Ruelke and D. E. Franks. 1975. Effect of Maturity and Protein Supplementation on Voluntary Intake and Nutrient Digestibility of Pangola Digitgrass Hays. J. Anim. Sci. 40:769-774.