Accumulation of Digestible Materials with Growth of Forages and Digestion of Forage Dry Matter by in vitro Incubation with Rumen Fluid and Pepsin

Shimojo, Masataka
Laboratory of Animal Feed Science, Faculty of Agriculture, Kyushu University

Bungo, Takashi
Laboratory of Animal Feed Science, Faculty of Agriculture, Kyushu University

Imura, Yoshimi
Laboratory of Animal Feed Science, Faculty of Agriculture, Kyushu University

Tobisa, Manabu
Laboratory of Animal Feed Science, Faculty of Agriculture, Kyushu University

他

https://doi.org/10.5109/24283

出版情報：九州大学大学院農学研究院紀要. 43 (3/4), pp.373-382, 1999-02. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **43** (3·4), 373–382 (1999)

Accumulation of Digestible Materials with Growth of Forages and Digestion of Forage Dry Matter by *in vitro* Incubation with Rumen Fluid and Pepsin

Masataka Shimojo, Takashi Bungo, Yoshimi Imura, Manabu Tobisa, Naoki Koga, Tao Shao, Muhammad Yunus, Yutaka Nakano*, Ichiro Goto, Mitsuhiro Furuse and Yasuhisa Masuda**

Laboratory of Animal Feed Science, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
(*Received October 30, 1998 and accepted November 6, 1998*)

The present study was conducted to suggest an equation to the analysis of forage dry matter digestion by *in vitro* incubation with rumen fluid and pepsin, and also to suggest an equation, using digestion rate, to the analysis of decreases in dry matter digestibility [DDMD] with growth of forages. Forage digestion characteristics were relative digestion rate of dry matter [RDRW], degradation rate of lignin [DRL] and dry matter digestion per unit degradation of lignin [DWDL]. This method was applied to two tropical forages to evaluate (1) RDRW using DRL and DWDL, and (2) DDMD using RDRW. Accumulation of digestible materials and DDMD using accumulation rate with forage growth (Shimojo *et al.*, 1998a, b, c, 1999) were also taken up and applied to the same tropical forages. Two different-type equations for DDMD suggested, roughly speaking, a sort of relationship between digestion characteristics of forage dry matter in ruminants and accumulation characteristics of digestible materials in forages.

INTRODUCTION

Ruminant animals digest forages, but not all the components are digested in the rumen. The amount of materials in forages that are digestible to rumen microbes are, therefore, of importance to the ruminant production from forages (Van Soest, 1982; Minson, 1990). How the digestible materials accumulate with growth of forages and how forage dry matter is digested by rumen microbes are considered the subject that attracts those people who are interested in forage–ruminant close relationships.

In our recent reports (Shimojo *et al.*, 1998a, b, c, 1999) a simple equation, whose construction was based on the method of forage growth analysis (Hunt, 1990), was suggested to the analysis of the accumulation of digestible materials with forage growth and was applied to some tropical forages. In those four papers the amount of digestible materials in forages was determined by *in vitro* incubation with rumen fluid and pepsin using the method of Minson and McLeod (1972). For evaluating the digestion or degradation characteristics of forages and fibrous feeds in the rumen, there are analytic methods using exponential equations, such as the method of Mertens and Loften (1980) and that of Ørskov (1989). In addition, there might be a possibility of applying growth analysis method, if modified appropriately, to the evaluation of digestion characteristics of forage dry matter. This approach might be expected to give the analytic viewpoint to

* Kyushu University Farm, Fukuoka 811–2307
** University of the Air, Fukuoka Study Center, Fukuoka 812–0016

both the digestion of forage dry matter by rumen fluid and pepsin and the accumulation of digestible materials with growth of forages.

The present study was designed to suggest a simple equation to the analysis of the digestion of forage dry matter by *in vitro* incubation with rumen fluid and pepsin, followed by suggesting a sort of relationship between digestion characteristics of forage dry matter and accumulation characteristics of digestible materials with growth of forages.

AN EQUATION FOR ANALYZING DIGESTION OF FORAGE DRY MATTER BY *IN VITRO* INCUBATION

An equation for analyzing forage dry matter digestion by *in vitro* incubation with rumen fluid and pepsin

In the present study the relative digestion rate of forage dry matter [RDRW] by *in vitro* incubation with rumen fluid and pepsin is taken up. RDRW is suggested as follows:

$$\mathrm{RDRW} = \frac{1}{W} \cdot \frac{dW}{dT}, \qquad (1)$$

where W=forage dry matter weight, T=time of *in vitro* incubation with rumen fluid followed by pepsin.

The dry matter that is digested by *in vitro* incubation is regarded as the digestible materials of forages.

Forage dry matter digestion is influenced mainly by lignin that partly protects plant fibers from the attack by rumen microbes and related enzymes (Van Soest, 1982; Minson, 1990; Cheng *et al.*, 1991). It was shown in the long-period *in vitro* incubation with rumen fluid that the digestion of dry matter advanced with the increase in the degradation of lignin (Goto and Minson, 1977). This was also found in the *in vitro* incubation with rumen fluid and pepsin, using the method of Minson and McLeod (1972), for forages (Shimojo and Goto, 1984, 1988, 1990b, c) and for cereal straws (Song *et al.*, 1991a, b, c). In those eight reports on the influence of lignin degradation on dry matter digestion, acetyl bromide lignin [ABL] (Morrison, 1972a, b) was used in place of acid detergent lignin [ADL] (Goering and Van Soest, 1970). ADL is widely accepted for the determination of lignin content of forages, and ABL might be considered suitable when the forage digestion is related to the lignin degradation. Also in our previous studies digestibility was related with ABL content for forages (Nakanishi *et al.*, 1985; Shimojo and Goto, 1987, 1990a, b, c) and for cereal straws (Song *et al.*, 1991a, b).

When ABL degradation is incorporated, equation (1) is rewritten as follows:

$$\mathrm{RDRW} = \frac{1}{W} \cdot \frac{dW}{dT}$$

$$= \left(\frac{1}{W} \cdot \frac{d(ABL)}{dT} \right) \cdot \frac{dW}{d(ABL)}, \qquad (2)$$

where ABL denotes its amount, $(1/W)\cdot(d(ABL)/dT)$=degradation rate of ABL [DRL] per unit W, $(dW/d(ABL))$=digestion of W per unit degradation of ABL [DWDL].

Thus, RDRW is expressed as the product of DRL and DWDL.

The mean value over the interval T_1 to T_2 in the *in vitro* incubation for each of RDRW, DRL and DWDL is approximately as follows:

$$\overline{\mathrm{RDRW}} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}, \tag{3}$$

$$\overline{\mathrm{DRL}} = \frac{\log_e W_2 - \log_e W_1}{W_2 - W_1} \cdot \frac{(ABL)_2 - (ABL)_1}{T_2 - T_1}, \tag{4}$$

$$\overline{\mathrm{DWDL}} = \frac{W_2 - W_1}{(ABL)_2 - (ABL)_1}, \tag{5}$$

where e=the base of natural logarithm.

It is usually recommended that $\overline{\mathrm{RDRW}}$, $\overline{\mathrm{DRL}}$ and $\overline{\mathrm{DWDL}}$ are determined at intervals of several hours in the incubation.

When the forage is incubated *in vitro* with rumen fluid for 2 days followed by pepsin for another 2 days (Minson and McLeod, 1972), the amount of digestible materials is described as the difference in W between before and after the 4-day incubation. Thus, if W before the incubation is termed W_0 and that after the 4-day incubation is termed W_4 ($W_0 > W_4$), dry matter digestibility [DMD] is as follows:

$$\mathrm{DMD} = -\left(\frac{W_4 - W_0}{W_0}\right)$$

$$= -\left(\frac{\Delta W}{W_0}\right). \tag{6}$$

Then, the following is given from equation (1),

$$dW = (\mathrm{RDRW}) \cdot (W) \cdot (dT) \tag{7}$$

Equation (7) is rewritten as follows when applied to over the 4-day incubation,

$$\Delta W = 4 \cdot (\overline{\mathrm{RDRW}}) \cdot (\overline{W}) \tag{8}$$

Inserting equation (8) into equation (6) gives

$$\mathrm{DMD} = -4 \cdot \left\{\frac{(\overline{\mathrm{RDRW}}) \cdot (\overline{W})}{W_0}\right\}. \tag{9}$$

As shown in equation (9), the calculation of DMD requires $\overline{\mathrm{RDRW}}$ over the 4-day incubation. Mean value over 4 days does not give detailed descriptions of the digestion process, when compared with $\overline{\mathrm{RDRW}}$ determined at intervals of several hours. It seems, however, that $\overline{\mathrm{RDRW}}$ over 4 days might give a broad outline of the digestion rate of forages.

If DMD of the forage cut at its growth time t_1 is termed DMD_1 and that at t_2 is termed

DMD_2, then the decrease in DMD [DDMD] with forage growth (from t_1 to t_2) is described as follows:

$$DDMD = DMD_1 - DMD_2$$

$$= \left[-4 \cdot \left\{\frac{(\overline{RDRW}) \cdot (\overline{W})}{W_0}\right\}\right]_1 - \left[-4 \cdot \left\{\frac{(\overline{RDWR}) \cdot (\overline{W})}{W_0}\right\}\right]_2$$

$$= -4 \cdot \left[\left\{\frac{(\overline{RDRW}) \cdot (\overline{W})}{W_0}\right\}_1 - \left\{\frac{(\overline{RDRW}) \cdot (\overline{W})}{W_0}\right\}_2\right]. \qquad (10)$$

Applications to two tropical forages

(A) Forages

Two tropical forages are Rhodes grass (*Chloris gayana* Kunth) and Greenleaf desmodium (*Desmodium intortum* (Mill.) Urb.). Rhodes grass [Rg] and Greenleaf desmodium [Gd] were cut at 35 and 63 days of regrowth with a compound fertilizer ($N:P_2O_5:K_2O$=14:14:14%) which had been dressed, immediately after the first cut and discard, at a rate of 1.0 kg/a for each element.

(B) Digestion of Rg by *in vitro* incubation

Digestion characteristics of Rg before and after *in vitro* incubation with rumen fluid

Table 1. Analyses of the digestion of dry matter of Rhodes grass [Rg] by *in vitro* incubation with rumen fluid of goats for 2 days followed by pepsin for 2 days.

Regrowth: t(days)	35 (A)		63 (B)		A/B
Incubation: T(days)	0	4	0	4	
Forage dry weight: W(g/m²)	225.56	81.36	515.00	257.87	
Amount of lignin: ABL(g/m²)	20.32	11.67	61.53	41.29	
$\overline{RDRW}=(\overline{DRL})\cdot(\overline{DWDL})$					
$\overline{RDRW}$ (g/g/day)		−0.2549		−0.1729	1.4742
$\overline{DRL}$ (g/g/day)		−0.0153		−0.0136	1.1234
$\overline{DWDL}$ (g/g)		16.6705		12.7041	1.3122
$DMD=-4\{(\overline{RDRW})\cdot(\overline{W})\}/W_0$					
DMD (g/g)		0.6393		0.4993	1.2804
$\overline{RDRW}$ (g/g/day)		−0.2549		−0.1729	1.4742
$\overline{W}$ (g)		141.41		371.73	0.3804
W_0 (g)		225.56		515.00	0.4380
$1/W_0$		0.0044		0.0019	2.2832
$DDMD=DMD_{35}-DMD_{63}$			0.1400		

ABL=acetyl bromide lignin, RDRW=relative digestion rate of W by *in vitro* incubation with rumen fluid and pepsin, DRL=degradation rate of *ABL* by the incubation, DWDL=digestion of W per unit degradation of *ABL*, DMD=dry matter digestibility, DDMD=the decrease in DMD during the regrowth, $\overline{W}$=mean W over the 4-day incubation, W_0=W before the incubation.

of goats and pepsin are shown in Table 1. $\overline{\text{RDRW}}$ was higher at 35 days than at 63 days of regrowth (−0.2549 versus −0.1729). This was mainly due to higher $\overline{\text{DWDL}}$ at 35 days (16.6705) compared with that at 63 days (12.7045) of regrowth, with some contribution of slightly higher $\overline{\text{DRL}}$ (−0.0153 versus −0.0136).

DMD and its equation's components and DDMD are also shown in Table 1. DMD at 35 days and that at 63 days of regrowth were 0.6393 and 0.4993, respectively, therefore, DDMD over the interval of 28 days was 0.1400. The higher DMD at 35 days than at 63 days was mainly due to higher $\overline{\text{RDRW}}$ (−0.2549 versus −0.1729), because lower $\overline{W}$ (141.41 versus 371.72) seemed to be offset by higher $1/W_0$ (0.0044 versus 0.0019).

It was suggested in Rg that the present method accounted analytically for (1) how forage dry matter digestion was influenced by lignin degradation, and also for (2) how the dry matter digestibility and its decreases with forage growth were related to the events in (1).

(C) Digestion of Gd by *in vitro* incubation

Digestion characteristics of Gd before and after *in vitro* incubation with rumen fluid of goats and pepsin are shown in Table 2. There was only a small difference in $\overline{\text{RDRW}}$ between 35 and 63 days of regrowth (−0.2036 versus −0.1885). This was due to slightly higher $\overline{\text{DWDL}}$ (13.7289 versus 13.0016), because $\overline{\text{DRL}}$ showed almost the same value (−0.0148 versus −0.0145).

DMD and its equation's components and DDMD are also shown in Table 2. DMD at

Table 2. Analyses of the digestion of dry matter of Greenleaf desmodium [Gd] by *in vitro* incubation with rumen fluid of goats for 2 days followed by pepsin for 2 days.

Regrowth: t(days)	35 (A)		63 (B)		A/B
Incubation: T(days)	0	4	0	4	
Forage dry weight: W(g/m²)	190.00	84.16	315.28	148.35	
Amount of lignin: ABL(g/m²)	22.46	14.75	39.67	26.83	
$\overline{\text{RDRW}}=(\overline{\text{DRL}})\cdot(\overline{\text{DWDL}})$					
$\overline{\text{RDRW}}$ (g/g/day)		−0.2036		−0.1885	1.0801
$\overline{\text{DRL}}$ (g/g/day)		−0.0148		−0.0145	1.0230
$\overline{\text{DWDL}}$ (g/g)		13.7276		13.0008	1.0559
$\text{DMD}=-4\{(\overline{\text{RDRW}})\cdot(\overline{W})\}/W_0$					
DMD (g/g)		0.5571		0.5295	1.0521
$\overline{\text{RDRW}}$ (g/g/day)		−0.2036		−0.1885	1.0801
$\overline{W}$ (g)		129.98		221.43	0.5870
W_0 (g)		190.00		315.28	0.6026
$1/W_0$		0.0053		0.0032	1.6594
$\text{DDMD}=\text{DMD}_{35}-\text{DMD}_{63}$			0.0276		

ABL=acetyl bromide lignin, RDRW=relative digestion rate of W by *in vitro* incubation with rumen fluid and pepsin, DRL=degradation rate of *ABL* by the incubation, DWDL=digestion of W per unit degradation of *ABL*, DMD=dry matter digestibility, DDMD=the decrease in DMD during the regrowth, $\overline{W}$=mean W over the 4-day incubation, W_0=W before the incubation.

35 days and that at 63 days of regrowth were 0.5571 and 0.5295, respectively, and DDMD over the interval of 28 days was 0.0276. The slightly higher DMD at 35 days than at 63 days was mainly due to slightly higher RDR$\overline{W}$ (−0.2036 versus −0.1885), because lower $\overline{W}$ (129.97 versus 221.42) seemed to be offset by higher $1/W_0$ (0.0053 versus 0.0032).

It was suggested in Gd that the present method accounted analytically for (1) the influence of lignin degradation on forage dry matter digestion, and for (2) how the dry matter digestibility and its decreases with forage growth were related to the events in (1).

To examine the present method, there is a necessity for applying it to various forages grown under different conditions.

AN EQUATION FOR ANALYZING ACCUMULATION OF DIGESTIBLE MATERIALS WITH GROWTH OF FORAGES

An equation for analyzing the accumulation of digestible materials with forage growth

We suggested, in reports (Shimojo *et al.*, 1998a, b, c, 1999), an equation to the analysis of the accumulation of digestible materials with growth of forages. In those four papers the amount of digestible materials was determined by *in vitro* incubation of forages with rumen fluid of goats and pepsin using the method of Minson and McLeod (1972), namely the method used in the previous section.

The accumulation rate of digestible materials [ARD] per unit dry weight of the forage with its growth is as follows:

$$\mathrm{ARD} = \frac{1}{W} \cdot \frac{dD}{dt}$$

$$= \left(\frac{D + \dfrac{dW}{Dt}}{W} \right) \cdot \left(\frac{1}{D + \dfrac{dW}{dt}} \cdot \frac{dD}{dt} \right), \qquad (11)$$

where W=forage dry weight, D=dry weight of digestible materials in the forage, t=days of forage growth, (dW/dt)=new photosynthates (expressed in weight, not in rate), $(D+dW/dt)$=source materials [S] for D accumulation, $(D+dW/dt)/W$=the ratio of S to W [S ratio], $[\{1/(D+dW/dt)\}\cdot(dD/dt)]$=accumulation rate of D per unit S [ARDS].

With forage growth dry matter digestibility [DMD] normally decreases even when there occurs the accumulation of D. The decrease in dry matter digestibility [DDMD] with forage growth is as follows:

$$\mathrm{DDMD} = \frac{D_1}{W_1} - \frac{D_2}{W_2}$$

$$= \left(1 - \frac{W_1}{W_2} \right) \cdot \left(\frac{D_1}{W_1} - \frac{\overline{\mathrm{ARD}}}{\overline{\mathrm{RGR}}} \right), \qquad (12)$$

where $\overline{\mathrm{ARD}}$=mean ARD over the interval t_1 to t_2, $\overline{\mathrm{RGR}}$=mean relative growth rate [RGR] of the forage over the interval t_1 to t_2, $(1-W_1/W_2)$=forage growth index [FG index], $\{D_1/W_1$

$-\overline{ARD}/\overline{RGR}$]=an index for the decrease in dry matter partition into digestible materials [DDMPD index].

The mean value over the interval t_1 to t_2 for each of ARD, S ratio, ARDS and RGR is approximately as follows:

$$\overline{ARD} = \frac{\log_e W_2 - \log_e W_1}{W_2 - W_1} \cdot \frac{D_2 - D_1}{t_2 - t_1}, \tag{13}$$

$$\overline{S\ ratio} = \left(\frac{D_2 - D_1}{\log_e D_2 - \log_e D_1} + \frac{W_2 - W_1}{t_2 - t_1} \right) \cdot \frac{\log_e W_2 - \log_e W_1}{W_2 - W_1}, \tag{14}$$

$$\overline{ARDS} = \frac{1}{\dfrac{D_2 - D_1}{\log_e D_2 - \log_e D_1} + \dfrac{W_2 - W_1}{t_2 - t_1}} \cdot \frac{D_2 - D_1}{t_2 - t_1}, \tag{15}$$

$$\overline{RGR} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}, \tag{16}$$

where e=the base of natural logarithm.

Applications to the two tropical forages

(A) Forages

The two tropical forages, Rhodes grass [Rg] and Greenleaf desmodium [Gd], are the

Table 3. Analyses of the accumulation of digestible materials with growth of Rhodes grass [Rg] and Greenleaf desmodium [Gd].

Forages	Rg		Gd		Rg/Gd
Regrowth: t(days)	35	63	35	63	
Forage dry weight: W (g/m^2)	225.56	515.00	190.00	315.28	
Dry weight of digestible materials in forage: D (g/m^2)	144.20	257.13	105.84	166.93	
Dry matter digestibility (g/g)	0.6393	0.4993	0.5571	0.5295	
Interval (days)	28		28		
$\overline{ARD}$=($\overline{S\ ratio}$)·($\overline{ARDS}$)					
$\overline{ARD}$ (g/g/day)	0.0115		0.0088		1.3044
$\overline{S\ ratio}$ (g/g)	0.5864		0.5601		1.0470
$\overline{ARDS}$ (g/g/day)	0.0196		0.0157		1.2458
DDMD=(FG index)·(DDMPD index)					
DDMD	0.1400		0.0276		5.0755
FG index	0.5620		0.3974		1.4144
DDMPD index	0.2491		0.0694		3.5885

RGR=forage relative growth rate, ARD=accumulation rate of D per unit W, S ratio=the ratio of source materials [S] ($S=D+dW/dt$) to W, ARDS=accumulation rate of D per unit S, DDMD=the decrease in dry matter digestibility during the regrowth, FG index=forage growth index, DDMPD index=an index for the decrease in dry matter partition into digestible materials.

same as used in the analysis of digestion characteristics in the previous section.

(B) $\overline{ARD}$ and DDMD for Rg and those for Gd over the growth of them

Forage characteristics, $\overline{ARD}$ and its components, and DDMD and its equation's components for Rg and those for Gd are shown in Table 3. In the present study, comparisons were made between Rg and Gd. $\overline{ARD}$ of Rg (0.0115) was higher than that of Gd (0.0088). This was mainly due to higher $\overline{ARDS}$ in Rg compared with Gd (0.0196 versus 0.0157), because $\overline{S\ ratio}$ showed only a small difference between the two forages (0.5864 versus 0.5601).

DDMD was larger in Rg than in Gd (0.1400 versus 0.0276). This was mainly due to higher DDMPD index of Rg (0.2491) compared with that of Gd (0.0694), with some contribution of FG index (0.5620 versus 0.3974).

It was suggested that this method accounted analytically for (1) how the digestible materials accumulated with forage growth and (2) how the decrease in dry matter digestibility was related to forage growth and dry matter partition into digestible materials. This was shown in previous reports (Shimojo *et al.*, 1998a, b, c, 1999), but there is a necessity for its further examining using various forages grown under different conditions.

DDMD EQUATION USING RDRW AND THAT USING ARD

In the present study two different-type equations for describing DDMD were suggested, namely DDMD equation using $\overline{RDRW}$ [equation (10)] and that using $\overline{ARD}$ [equation (12)]. Thus, the following relationship is given

$$-4\cdot\left[\left\{\frac{\left(\overline{RDRW}\right)\cdot\left(\bar{W}\right)}{W_0}\right\}_1-\left\{\frac{\left(\overline{RDRW}\right)\cdot\left(\bar{W}\right)}{W_0}\right\}_2\right]=\left(1-\frac{W_1}{W_2}\right)\cdot\left\{\frac{D_1}{W_1}-\frac{\overline{ARD}}{\overline{RGR}}\right\}. \qquad (17)$$

The left-hand side of equation (17) accounts for how DDMD is related to $\overline{RDRW}$ given by the *in vitro* incubation of forages with rumen fluid and pepsin, and the right-hand side accounts for how DDMD is related to $\overline{ARD}$ with growth of forages. This suggests, roughly speaking, a sort of relationship between forage digestion characteristics in ruminants and accumulation characteristics of digestible materials in forages through digestibility changes with growth of forages. It also seems that equation (17), as it includes forage $\overline{RGR}$ in the right-hand side, analyzes forages from the viewpoint of both production and utilization characteristics.

Anyway, the two methods taken up in the present study should be applied to various cases.

Conclusions

Simple equations were suggested to the analysis of forage digestion by *in vitro* incubation with rumen fluid and pepsin and to the description of digestibility decreases with forage growth. The digestion characteristics of forage dry matter was related to the degradation characteristics of lignin. Forage dry matter digestion characteristics were

related with accumulation characteristics of digestible materials in forages through two different-type equations describing digestibility decreases with growth of forages.

ACKNOWLEDGEMENTS

We wish to thank Mr. Yasukatsu Yano for his skilled technical assistance in the present work.

REFERENCES

Cheng, K. -J., C. W. Forsberg, H. Minato and J. W.Costerton 1991 Microbial ecology and physiology of feed degradation within the rumen. *In* "Physiological Aspects of Digestion and Metabolism in Ruminants ", ed. by T. Tsuda *et al.*, Academic Press, Inc., San Diego, pp. 595–624

Goering, H. K. and P. J. Van Soest 1970 Forage fiber analyses. *Agriculture Handbook (U.S.D.A.)*, **No. 379**: 1–20

Goto, I. and D. J. Minson 1977 The potential digestibility of leaf and stem fractions of grasses. *J. Agric. Sci., Camb.*, **89**: 143–149

Hunt, R. 1990 *Basic Growth Analysis*. Unwin Hyman Ltd., London, U. K.

Mertens, D. R. and J. R. Loften 1980 The effect of starch on forage fiber digestion kinetics *in vitro*. *J.Dairy Sci.*, **63**: 1437–1446

Minson, D. J. 1990 Digestible energy of forage. *In* "Forage in Ruminant Nutrition", Academic Press, Inc., San Diego, pp. 85–149

Minson, D. J. and M. N. McLeod 1972 The *in vitro* technique: Its modification for estimating digestibility of large numbers of tropical pasture samples. *C.S.I.R.O. Div. Trop. Pastures Tech.Pap.*, **No. 8**: 1–15

Morrison, I. M. 1972a A semi-micro method for the determination of lignin and its use in predicting the digestibility of forage crops. *J. Sci. Food Agric.*, **23**: 455–463

Morrison, I. M. 1972b Improvements in the acetyl bromide technique to determine lignin and digestibility and its application to legumes. *J. Sci. Food Agric.*, **23**: 1463–1469

Nakanishi, Y., M. Shimojo and I. Goto 1985 Effect of growing stages of Rhodes grass on ingestive behaviour of grazing goats. *Sci. Bull. Fac. Agr., Kyushu Univ.*, **40**: 33–38 (in Japanese with English summary)

Ørskov, E. R. 1989 Recent advances in evaluation of roughages as feeds for ruminants. *In* "Recent Advances in Animal Nutrition in Australia 1989", ed. by D. J. Farrell, University of New England Printery, Armidale, pp. 102–108

Shimojo, M. and I. Goto 1984 A study on the relation between disappearance of dry matter and acetyl bromide lignin of tropical grass. *Jpn. J. Zootech Sci.*, **55**: 838–842 (in Japanese with English summary)

Shimojo, M. and I. Goto 1987 A study on the relation between organic matter disappearance and acetyl bromide lignin of tropical forages. *Jpn. J. Zootech Sci.*, **58**: 750–753 (in Japanese with English summary)

Shimojo, M. and I. Goto 1988 A study on the relation between organic matter and acetyl bromide lignin disappearances in tropical legumes. *Sci. Bull. Fac. Agr., Kyushu Univ.*, **42**: 153–156 (in Japanese with English summary)

Shimojo, M and I. Goto 1990a Improvement of nutritive value of tropical grasses by physical or chemical treatment. 1. Effect of steam treatment on chemical composition and dry matter digestibility. *J. Japan. Grassl. Sci.*, **36**: 184–190 (in Japanese with English summary)

Shimojo, M and I. Goto 1990b Improvement of nutritive value of tropical grasses by physical or chemical treatment. 2. Effect of wet treatment with sodium hydroxide on chemical composition and dry matter digestibility. *J. Japan. Grassl. Sci.*, **36**: 191–196 (in Japanese with English summary)

Shimojo, M and I. Goto 1990c Improvement of nutritive value of tropical grasses by physical or chemical treatment. 3. Effect of ammonia treatment on chemical composition and dry matter digestibility. *J. Japan. Grassl. Sci.*, **36**: 197–202 (in Japanese with English summary)

Shimojo, M., T. Bungo, M. Tobisa, Y. Imura, N. Koga, S. Tao, M. Yunus, Z. Yin, Y. Nakano, I. Goto and Y.

Masuda 1998a Accumulation of digestible materials and decrease in dry matter digestibility in the growth of two tropical grasses. *J. Fac. Agr., Kyushu Univ.*, **42**: 365–372

Shimojo, M., T. Bungo, S. Tao, Y. Imura, M. Tobisa, N. Koga, M. Yunus, Y. Nakano, I. Goto, M. Furuse and Y. Masuda. 1998b Analyses of accumulation of digestible materials and decrease in dry matter digestibility with growth of Rhodes grass. *Proc. 8th World Conf. Anim. Prod.*, Seoul, Korea, **Vol. II**: 516–517

Shimojo, M., T. Bungo, Y. Imura, M. Tobisa, N. Koga, S. Tao, M. Yunus, Y. Nakano, I. Goto, M. Furuse and Y. Masuda 1998c Accumulation rate of digestible materials and formation rate of indigestible materials in the description of relative growth rate of forages. *J. Fac. Agr., Kyushu Univ.*, **43**: 119–126

Shimojo, M., T. Bungo, Y. Imura, M. Tobisa, N. Koga, T. Shao, M. Yunus, Y. Nakano, I. Goto, M. Furuse and Y. Masuda 1999 Two different-type equations of relative growth analysis for both forages and ruminants and deriving of them from a hypothetic equation. *J. Fac. Agr., Kyushu Univ.*, **43**: in press

Song, Y. H., M. Shimojo and I. Goto 1991a Improvement of nutritive value of rice straw by treatment with ammonia and sulphur dioxide. *J. Fac. Agr., Kyushu Univ.*, **36**: 143–149

Song, Y. H., M. Shimojo and I. Goto 1991b Improvement of nutritive value of barley straw by treatment with ammonia and sulphur dioxide. *J. Fac. Agr., Kyushu Univ.*, **36**: 151–156

Song, Y. H., M. Shimojo and I. Goto 1991c Effect of sulphur dioxide treatment on the quality of wheat straw pretreated with ammonia. *J. Fac. Agr., Kyushu Univ.*, **36**: 157–164

Van Soest, P. J. 1982 Nutritional quality. *In* "Nutritional Ecology of the Ruminant", Cornell University Press, New York, pp. 23–74