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Relationship between Voluntary Intake of Timothy Hay in Dairy Cattle and Chemical Composition, Retention Time in the Rumen, Digestibility and Digestion Rates

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Abstract

Three experiments were carried out to study the effects of feed characteristics on voluntary intake that was measured as dry matter intake (DMI) using 4 dairy cows offered timothy hay *ad libitum*. The digestibility depending on the chemical composition of timothy hay was determined using the total collection method in sheep, while the rate of fiber degradation in the rumen was calculated by the *in vitro* method. Mean daily DMI and DMI per metabolic body weight of 4 types of timothy hay ranged from 6.5 to 9.1 kg/day, and 56.9 to 78.8 g/kg$^{0.75}$, respectively. The daily DMI of timothy hay by dairy cows was closely related to the organic b fraction (Ob), sum of organic cellular contents and organic a fraction (OCC+Oa), and content of acid detergent lignin (ADL). The daily DMI could be predicted from the following regression equations; DMI = –0.18 × Ob + 19.5 (r=0.993), DMI = –0.50 × ADL + 12.1 (r=0.997), DMI = 0.19 × (OCC+Oa) –1.6 (r=0.993). In addition, the daily DMI of timothy hay by dairy cows was also closely related to the retention time in the rumen, digestibility depending on the chemical composition and rate of degradation of fibers in the rumen.

Discipline: Animal industry
Additional key words: feed, dry matter intake, animal nutrition

Introduction

Average milk production in Japan has increased, reaching 8,300 kg per head[13], due to the introduction to the feeding management for dairy cattle of a new index of feeding management, i.e. fibrous fractionation based on dietary fiber image or nonstructural carbohydrates and protein fractionation based on the solubility in the rumen. Recently, the nutritive value of feed in ruminants based on the fibrous composition of forages has been evaluated by the enzymatic method or detergent method, in addition to the proximate method. Nutritive value of forage such as total digestible nutrients (TDN) is generally calculated by using a regression equation based on the chemical composition. These methods are widely used for feeding ration planning. However, the apparent feed intake is more important for maintaining the milk production. Especially, for feeding management of dairy cattle for high milk production, the proportion of concentrates is relatively limited compared to that of forage, due to the adverse effect of an excessive amount of concentrates. Moreover the processing of the forage in the form of silage or hay affects the intake of forage.

Therefore, the objective of this study was to predict the dry matter intake of timothy hay used in Japan, based on the relationship between the dry matter intake and chemical composition, retention time in the rumen, digestibility and digestion rate.

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Materials and methods

Four types of timothy hay at the first cutting were used in 3 experiments for studying (1) voluntary feed intake, (2) *in vivo* and (3) *in vitro* digestibility. Two compact baled hays were imported from the USA, and another one was prepared in Hokkaido.

1) Experiment 1 Voluntary intake of timothy hay

(1) Animals and feeding

Four Holstein cows (2–4 years of age) in the dry period with a body weight ranging from 460 to 628 kg were used. Voluntary feed intake was measured as dry matter intake (DMI) in cows offered hay *ad libitum*. Cows were allowed 3 days to adapt to the experimental feeding and environment prior to the 7-day collection period. Timothy hay was supplied once daily at 9:00 am at the rate of 10–15 kg to obtain feed residues. Dry matter intake was determined by measuring the amount of daily residues.

(2) Chemical composition

The 4 types of timothy hay were analyzed for the moisture content, organic matter (OM), crude protein (CP) and ether extracts (EE) contents by a proximate analysis method[12]. Fibrous fractions were also measured using the enzymatic method[2,3] and detergent method[14,15]. The enzymatic method was used for determining organic cellular contents (OCC), organic cell wall (OCW) content, fibers with high digestibility (Organic a fraction: Oa) content, and fibers with low digestibility (Organic b fraction: Ob) content, while the detergent method was used for the determination of the neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents.

(3) Measurement of retention time in the digestive tract

The retention time was measured by the addition of Ytterbium (Yb) to 500 g of homogeneous timothy hay that was then fed to dairy cows prior to allowing the experimental feeding, according to the method described by Cameron et al.[5]. Measurement of the retention time in the digestive tract was conducted from day-2 of the collection period. Fecal samples were collected at 4, 8, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96, 120 h after administration, and the concentration of Yb was determined by atomic absorption spectrophotometry (AA-6400 series, Shimadzu Co, Japan). Total retention time was measured in the whole digestive tract and in each part, i.e. in the rumen, in the lower digestive tract and in the intestine by calculation of the Yb concentration in feces using the method described by Hidari et al.[7].

(4) Rumen juice

The rumen juice was taken on the last day of the collection period, and was then analyzed for determining the pH (by pH meter), ammonia nitrogen content (Kjeldahl method) and content of volatile fatty acids (VFAs, by gas chromatography), to evaluate the performance of fermentation in the rumen.

2) Experiment 2 In vivo *digestion trial of timothy hay*

The digestibility depending on the chemical composition of each type of timothy hay was determined by the digestion trial using 12 castrated Corriedale sheep. The experiment was carried out by one-way layout classification[11] and 3 sheep were allocated to each hay sample. Preliminary digestion trials were allowed for 7 days following 6 days of total collection period. In this experiment, soybean meal was added to timothy hay to reach a CP content of feed of 12% (dry matter basis). Timothy hay was chopped and given as fragments, 7 cm long. The feed was administered once a day at 10:00 am and supplied at a rate of 1.5% of the body weight to minimize the amount of residues. The method employed for fecal analysis followed that used for feed analysis of timothy hay.

3) Experiment 3 In vitro *digestion trial of timothy hay*

The *in vitro* digestion trial of the 4 types of timothy hay was carried out by applying the carbon dioxide flowing-gas technique[1,8]. The rumen juice for culture was collected from the cattle used in the voluntary intake experiment (Exp. 1). The duration of the culture period for the *in vitro* digestion trial was 2, 4, 6, 8, 12, 24, 48, 72 h, with a degradation rate estimated at 72 h of culture for the potential digestibility of timothy hay. For each period of culture, the pH of the culture solution was measured, and the digestibility and digestion rate of DM and OCW were calculated by the method of Mertens et al.[10].

Results and discussion

1) Voluntary intake of timothy hay

(1) Chemical composition and voluntary feed intake

Table 1 shows the chemical composition and voluntary intake of the 4 types of timothy hay used in the experiment. The values of the major components in timothy hay were 71.4–80.6% for OCW, 5.7–8.4% for CP, and 5.9–11.1% for ADL, respectively. The daily dry matter intake (DMI) for hays A, B, C, and D was 9.1, 7.9, 8.3, and 6.5 kg/day, respectively, while for the DMI per metabolic body size (MDMI) of these hays, the values were 78.8, 68.6, 71.7 and 56.9 g/kg$^{0.75}$, respectively. Timothy hay that contained a large amount of OCW tended to show a lower DMI, or in other words, the decrease of the DMI and MDMI was related to the progression of the

Table 1. Chemical composition and voluntary dry matter intake of timothy hay

	Hay A	Hay B	Hay C	Hay D
Chemical composition (DM%)				
Crude protein	8.4	5.7	7.5	6.6
Crude fat	2.0	1.7	1.5	1.3
Organic matter	93.9	93.7	94.5	94.6
Organic cellular contents	20.3	18.5	23.2	14.0
Organic cell wall	73.6	75.3	71.4	80.6
Fiber fraction with high digestibility	17.4	14.2	11.7	10.8
Fiber fraction with low digestibility	56.2	61.1	59.7	69.8
Neutral detergent fiber	70.8	72.5	71.4	79.6
Acid detergent fiber	40.4	43.7	39.8	47.9
Acid detergent lignin	5.9	8.1	7.7	11.1
Voluntary intake				
Dry matter intake (kg/day)	9.1	7.9	8.3	6.5
DMI per metabolic body size (g/kg$^{0.75}$)	78.8	68.6	71.7	56.9

DMI: Dry matter intake.

Table 2. Relationship between voluntary intake of timothy hay in dairy cattle and chemical composition

Equation		r	Se
DMI per metabolic body size (g/kg$^{0.75}$)	= –1.97 × OCW + 217.7	–0.850	5.89
	= –1.56 × Ob + 165.6	–0.992	1.37
	= –2.11 × NDF + 224.7	–0.945	3.65
	= –2.25 × ADF + 165.9	–0.920	4.38
	= –4.22 × ADL + 103.6	–0.997	0.73
	= 1.63 × (OCC + Oa) – 15.9	0.992	1.40
Dry matter intake (kg/day)	= –0.23 × OCW + 25.8	–0.857	0.68
	= –0.18 × Ob + 19.5	–0.993	0.15
	= –0.50 × ADL + 12.1	–0.997	0.09
	= –0.25 × NDF + 26.6	–0.948	0.42
	= –0.27 × ADF + 19.6	–0.925	0.50
	= 0.19 × (OCC + Oa) – 1.6	0.993	0.15

DMI: Refer to Table 1, r: Correlation coefficient, Se: Standard error, OCW: Organic cell wall, Ob: Fiber fraction with low digestibility, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, OCC: Organic cellular contents, Oa: Fiber fraction with high degestibility.

growth stages due to the increase in the amount of OCW. The relationship between the DMI or MDMI and the chemical composition of timothy hay is shown in Table 2. The correlation coefficients (r) between DMI or MDMI and the chemical composition were highly negative and ranged from –0.850 to –0.997, respectively. The remarkably high correlation between Ob and ADL with the DMI or MDMI, can be ascribed to the fact that the quantity of indigestible fraction or fractions with a low digestibility like ADL or Ob directly affected the intake[6] due to the accumulation effect on the fullness of the rumen. The same phenomenon may account for the high correlation between the DMI or MDMI and OCW, NDF as well as ADF, which contained ADL whose content proportionally increased along with the growth stages. Meanwhile, there was a highly positive linear correlation between the DMI or MDMI with the digestible fraction (OCC+Oa) in contrast to the fraction with a low digestibility (OCW). These facts suggest that the content of fiber components in feeds is one of the factors controlling voluntary intake, and, therefore, the DMI and MDMI of timothy hay can be estimated from the content of fiber components.

(2) Retention time in the digestive tract

Table 3 shows the retention time of the 4 types of timothy hay in the digestive tract. The retention time in the whole digestive tract, in the rumen, in the lower digestive tract and transit time in the intestine were in the range of 75.4–92.2, 45.2–58.9, 18.6–21.6 and 10.9–12.2 h, respectively. Table 4 shows the relationship between

Table 3. Retention time of timothy hay by dairy cattle

(h)

	Hay A	Hay B	Hay C	Hay D
Retention time in whole digestive tract	77.3	84.8	75.4	92.2
Retention time in the rumen	45.2	51.3	45.9	58.9
Retention time in the lower digestive tract	20.4	21.3	18.6	21.6
Transit time in intestine	11.7	12.2	10.9	11.7

Table 4. Relationship between retention time in the rumen of timothy hay in dairy cattle and chemical composition

Equation		r	Se
Retention time in the rumen (h)	= 1.55 × OCW – 66.0	0.958	2.2
	= 1.06 × Ob – 14.8	0.964	2.0
	= 1.49 × NDF – 59.4	0.961	2.1
	= 1.69 × ADF – 22.3	0.993	0.9
	= 2.78 × ADL + 27.6	0.945	2.5
	= –1.11 × (OCC + Oa) + 86.7	–0.978	1.6
DMI per metabolic body size ($g/kg^{0.75}$)	= –1.38 × RT + 138.5	–0.959	3.16
Dry matter intake (kg/day)	= –0.16 × RT + 16.3	–0.962	0.36

DMI: Refer to Table 1. r, Se, OCW, OCC, Oa, Ob, NDF, ADF, ADL: Refer to Table 2.
RT: Retention time in the rumen (h).

Table 5. Chemical composition of rumen juice in dairy cattle

	NH_3-N	VFA (Partial molar quantity)						Total acids	A/P
	(mg/dL)	Acetic	Propionic	Butyric	i-Valeric	Valeric	Caproic	(mol%)	
Hay A	5.8	68.3	20.3	8.4	1.5	0.8	0.6	47.7	3.4
Hay B	3.4	68.1	20.6	8.2	1.3	0.9	0.9	45.1	3.3
Hay C	5.7	68.6	20.7	7.7	1.4	0.8	0.8	49.3	3.3
Hay D	2.9	70.9	20.5	6.8	1.3	0.2	0.5	38.5	3.5

NH_3-N: Ammonia nitrogen (mg/dL), A/P: Rate of acetic acid and propionic acid.

the retention time in the digestive tract and the constituents of timothy hay. There was a high correlation between the retention time in the rumen and the constituents of timothy. The r and Standard error (Se) values for the correlation between the retention time in the rumen and fibrous components were 0.95–0.99 and 0.9–2.5, respectively. The values for the correlation with OCC+Oa were highly negative, being –0.98 and 1.6, respectively. Table 4 shows that the retention time of timothy in the rumen of dairy cattle could be estimated from the composition of fibrous fractions determined by enzymatic and detergent analytical methods. Significant correlation coefficients between the retention time and DMI or MDMI were observed only in the rumen, with a highly negative value of –0.96.

In this experiment, Yb was used as a marker for the passage time of the solid part of feed. The peak of Yb concentration appeared at about 30 and 48 h after feeding for hays A and D, respectively. The fastest (hays A and C) and the latest (hay D) peak appearance of Yb concentration for the 4 types of timothy hay differed by about 18 h. Therefore, the passage time in the rumen was considered to correspond to the passage time of the total fiber content in feed. As a result, it was considered that the amount of total fiber in feed is one of the factors influencing the passage time in the rumen, which in turn affected the DMI or MDMI.

(3) Rumen parameters

Table 5 shows the chemical components of rumen juice from cows fed 4 types of timothy hay. The pH of the rumen juice averaged 6.56, 6.94, 6.75 and 6.95 for hays A, B, C and D, respectively. The concentration of ammonia nitrogen (NH_3-N) of these hays was 5.8, 3.4, 5.7, 2.9 mg/dL for hays A, B, C and D, respectively. The amount of NH_3-N of hays B and D was lower than that of hays A and C. For the VFAs, the values of butyric acid, valeric acid, caproic acid of hay D were the lowest. Total acid quantity (mol%) of hay D was the lowest compared

Table 6. Relationship between voluntary intake of hay in dairy cattle and digestibility depending on chemical composition in *in vivo* digestion trial in sheep

	Digestibility (%)				DMI (kg/day)		DMI per metabolic body size (g/kg$^{0.75}$)	
	Hay A	Hay B	Hay C	Hay D	r	Se	r	Se
DM	66.9	62.8	63.1	46.4	0.958	3.21	0.960	0.37
OM	68.4	65.0	65.2	48.6	0.949	3.53	0.951	0.41
OCW	69.5	65.3	64.8	49.7	0.970	2.74	0.970	0.32
OCC	65.0	65.0	66.9	43.2	0.873	5.45	0.878	0.64
Oa	98.7	94.1	99.6	88.9	0.913	4.57	0.918	0.53
Ob	60.3	58.6	57.9	43.6	0.928	4.16	0.931	0.49
OCC+Oa	80.6	77.6	77.9	63.1	0.949	3.53	0.951	0.41
NDF	73.2	69.1	69.2	55.7	0.968	2.80	0.969	0.33
ADF	70.7	67.4	64.8	52.4	0.945	3.66	0.945	0.44
ADL	8.3	16.2	27.0	21.5	–0.513	9.60	–0.501	1.15
EE	46.0	39.9	36.5	26.4	0.953	3.38	0.951	0.41
CP	50.1	25.2	43.1	19.0	0.915	4.50	0.917	0.53
TDN	69.6	65.9	65.9	49.0	0.952	3.41	0.954	0.40

DMI: Refer to Table 1. r, Se, OCW, OCC, Oa, NDF, ADF, ADL: Refer to Table 2.
OM: Organic matter, EE: Ether extract, CP: Crude protein, TDN: Total digestible nutrients.

to the other types of hay. The ratio of acetic and propionic acids (the A/P ratio) in the 4 types of timothy hay was similar. Since fermentation of feed in the rumen led to a decrease of the pH due to the increase of the amounts of NH_3-N and VFAs from the decomposition of OCC or/and OCW in feed, the pH, NH_3-N and VFAs values in the rumen juice could be used as an index of feed decomposition. This may account for the lower pH and higher NH_3-N and VFAs values of hays A and C due to the decomposition associated with a higher DMI. On the other hand, the pH and NH_3-N values in hays B and D were higher and lower compared to those of hays A and C, respectively. Table 5 shows that feed decomposition for hay D was the lowest based on the high pH and low NH_3-N and VFAs values in the rumen juice.

Therefore, it was considered that the pH values, NH_3-N and VFAs contents of the rumen juice could be used as indices of DMI, because they reflected the feed decomposition amount and rate.

(4) Relationship among DMI, chemical composition and retention time

The results obtained indicated that the DMI or MDMI of timothy hay showed a high correlation with the chemical composition, especially OCC+Oa, ADL and fibrous component contents, mainly Ob. In addition, the results also showed that the DMI and MDMI were highly correlated with the retention time in the rumen. The content of total fiber in feed for which the digestion rate is comparatively slow seemed to be an important factor for the control of the DMI and MDMI in terms of the physical effect on the degree of distention of the rumen and rumen capacity. This fact was evident based on the relationship between the changes in the Yb concentration in feces and DMI and rumen parameters of timothy. Therefore, it can be considered that the chemical composition, the retention time and their interaction determine the DMI or MDMI.

2) In vivo *digestion trial in sheep*

Table 6 shows the digestibility of each component of timothy hays A, B, C and D in sheep. The dry matter digestibility of hays A, B, C ranged from 63 to 67%, but was lower for hay D, being 46%. The digestibility of OCW, OCC and Ob for hays A, B and C was in the range of 65–70, 65–67 and 58–60%, respectively. Meanwhile the corresponding values for hay D were 50, 43 and 44%, respectively. However, the digestibility of Oa for the 4 types ranged from 89 to 100%. A large difference in digestibility among the 4 types of hay was observed for ADL, CP, and EE. The TDN values which were 70% for hay A, 66% for hays B and C, and 49% for hay D, respectively, were highly correlated with the DMI or MDMI, as shown by the correlation coefficient of 0.95. The relationship between the DMI or MDMI and the digestibility of OCW and NDF was highly correlated, as shown by the correlation coefficients of 0.97. For the other components, high correlation coefficients were also obtained ranging from 0.87 to 0.96 with a small standard error ranging from 3.2 to 5.5. The results obtained in this study were in agreement with those obtained in a previous study[9], indicating that the DMI was determined by the digestibility.

Table 7. Digestion rate and lag time of DM and OCW in hay determined in *in vitro* trial

	Dry matter		Organic cell wall		
	k	r	k	lag time (h)	r
Hay A	0.083	0.997	0.088	1.42	0.996
Hay B	0.068	0.998	0.069	1.34	0.996
Hay C	0.058	0.996	0.055	1.07	0.996
Hay D	0.042	0.996	0.042	1.32	0.994

k: Digestion rate. r: Refer to Table 2.

Table 8. Relationship between voluntary intake of hay in dairy cattle and digestibility of dry matter and organic cell wall in *in vitro* trial

	Digestibility (%)				DMI (kg/day)		DMI per metabolic body size (g/kg$^{0.75}$)	
	Hay A	Hay B	Hay C	Hay D	r	Se	r	Se
Dry matter	64.3	59.6	61.6	52.7	0.995	0.13	0.994	1.24
Organic cell wall	57.8	52.3	53.6	49.1	0.972	0.31	0.974	2.51

DMI: Refer to Table 1. r, Se: Refer to Table 2.

3) In vitro *digestion trial*

The constant of digestion rate (k) and the lag time of decomposition in the rumen of dry matter and OCW are shown in Table 7. The k value of OCW was markedly different among the types, i.e. 0.088, 0.069, 0.055 and 0.042 for hays A, B, C and D, respectively. However, the lag time among the types was similar and ranged from 1.07 to 1.42 h. The large amount of DMI per day in hay A was rapidly digested, whereas the small amount of DMI per day in hay D was slowly digested. The relationship between the DMI or MDMI and the k value in timothy hay was expressed as correlation coefficients with the value of 0.88, which was lower than the value of the correlation between the DMI and feed composition or retention time in the rumen recorded in Experiment 1. The potential digestibility of timothy hay in the *in vitro* digestion trial determined in the 72-h culture is shown in Table 8. The values of the potential digestibility of dry matter and OCW were 60–64 and 52–58% for hays A, B and C, respectively. These values were lower in hay D, being 53 and 49%, respectively. Despite the fact that the correlation between the DMI or MDMI and the digestion rate was high, it was found that the potential digestibility determined in the 72-h *in vitro* culture may not be sufficient to characterize the digestibility, where other factors such as the fullness of the rumen and the animal requirement of feed were involved in the digestion rate. Moreover, the digestion rate of fibers was also considered to be an important factor for the control of the retention time or the passage time in the rumen, since the digestion rate of feed is markedly dependent on the amount of total fiber[4] that is rapidly decomposed after passage in the rumen.

The potential digestibility of dry matter and OCW determined in the digestion trial in sheep was higher than that in the *in vitro* trial. However, the correlation coefficients between the digestibility of dry matter or OCW *in vitro* and amount of DMI recorded in Experiment 1 were high, more than 0.97. Similar values were obtained in the correlation between the digestibility value obtained from the digestion trial using sheep and the *in vitro* trial. This study, therefore, confirmed that the values of digestibility *in vitro* can be used to determine the apparent digestibility in sheep.

Conclusion

The results of this study indicated that the feed composition, digestion rate, digestibility and retention time in the rumen are the factors that determine the DMI or MDMI which can be manipulated with the total fiber content in the rumen. This study, therefore, showed that timothy hay at the early growth stage exhibits a high nutritive value based on the performance of the DMI or MDMI, the composition, retention time, digestibility and digestion rates of total fiber and TDN determined in the *in vivo* and *in vitro* digestion trials. Thus, to obtain a high DMI, the animals should receive a feed with a low fiber content. Moreover, using the equations developed in the present study, the DMI or MDMI of timothy grass as well as of temperate grass could be estimated with considerable accuracy based on the Ob and ADL contents.

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