Comparison of *In Vitro* Digestion Kinetics of Cup-Plant and Alfalfa

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ABSTRACT : *In vitro* true digestibility of cup-plant (*Silphium perfoliatum* L.) is higher than other alternative forages and comparative to alfalfa (*Medicago sativa* L.) even at the high neutral detergent fiber (NDF) concentration. This study was conducted to determine whether the digestion kinetic parameters of cup-plant could explain high *in vitro* true digestibility of cup-plant at the several NDF levels. Cup-plant and alfalfa were both collected in Arlington and Lancaster, Wisconsin to meet the NDF content within 40 to 50% range. The collected samples were incubated with rumen juice to investigate the digestion kinetics at 3, 6, 9, 14, 20, 28, 36, 48, and 72 h. Kinetics was estimated by the model $R = D_0$ e-k(t-L)+U where R is residue remaining at time t, and D_0 is digestible fraction, k is digestion rate constant, L is discrete lag time, and U is indigestible fraction. Parameters of the model were estimated by the direct nonlinear least squares (DNLS) method. Digestion rate and potential extent of digestion were not statistically different in either forage. However, alfalfa had shorter lag time ($p<0.05$). The indigestible fraction increased with maturation in alfalfa and in cup-plant ($p<0.05$). The ratio of indigestible fraction to acid detergent lignin (ADL) was higher in cup-plant than in alfalfa ($p<0.05$). From the results, alfalfa is probably digested more rapidly than cup-plant, however, cup-plant maintains higher digestibility with maturation due to a relatively slower increase of indigestible fraction in NDF. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 5 : 641-644*)

Key Words : *Silphium perfoliatum* L., Digestion Rate, Neutral Detergent Fiber, Indigestible Fraction, Digestion Kinetic Parameters

INTRODUCTION

Cup-plant (*Silphium perfoliatum* L.) has proven its potential as an alternative forage with equivalent dry matter yield to corn or alfalfa, and less changeable forage quality by maturation. According to Albrecht and Bures (1999), the concentration of acid detergent fiber (ADF) and NDF in cup-plant was less changed from bud to mid-flower stage, moreover, *in vitro* true digestibility was high with the relatively high levels of ADF and NDF. Therefore, Albrecht and Bures (1999) concluded that cup-plant can present a broad window of harvest schedule to farmers.

Neutral detergent fiber (NDF) content was reported to have negative correlation with potential extent of digestibility (Jung et al., 1998). Therefore, relatively high *in vitro* digestibility of cup-plant at high NDF level can be regarded as special characteristics. First order reaction kinetics can interpret forage digestion model in the different forage maturity, composition and rate of fiber digestion (Smith et al., 1972). Bermudagrass also has been known to maintain high digestibility even at the high NDF concentration. Mertens and Loften (1980) observed that the cell walls in burmudagrass had higher digestion rate and higher potential extent of digestion than alfalfa even with longer lag time.

Moore and Cherney (1986) observed the similar digestion rate in several grass species like tall fescue, sorghum×sudangrass, and wheat straw. However, the differences of digestion parameters could reflect the various digestion models in forages. Degradability of fiber in legumes was faster, but the digestibility was lower than those in grasses (Smith et al., 1972). Buxton (1989) reported that digestion rate of potential digestible cell wall (PDCW) was 50% faster in legume than in grass stems and 70% faster in immature than in mature stems.

Maturation affected the digestion kinetic parameters through the physically and chemically altered cell walls. Twidwell et al. (1988) observed low digestibility with the progress of maturation in switchgrass. He took declined digestion rate as a reason for the low digestibility in matured plant.

Differences of digestion rate and extent of digestion were known to be affected by concentration or distribution of lignin in cell walls. Smith et al. (1971) observed the similar lignin-cellulose ratio in legumes and grasses. Cherney et al. (1986) observed that lignin concentration in residue of brown mid-rib sorghum and normal species merged into 138 g/kg as digestion progressed, thus, they concluded that lignin was the main factor that determined the extent of digestibility in the forage species. When comparing digestion kinetics of alfalfa, the following hypotheses can be considered in cup-plant: 1) shorter lag time 2) higher digestion rate 3) higher potential extent of digestion 4) less effect of lignin on indigestible

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fraction.

The objective of this experiment was to determine whether those hypotheses affect the cup-plant digestion.

MATERIALS AND METHODS

Sample preparation

Cup-plant samples were collected from the cup-plant field in Arlington, and Lancaster, WI. The *in vitro* digestion study was conducted at the Univ. of Wisconsin, Madison, WI. Alfalfa and cup-plant samples were collected to meet the 40 to 50% range of NDF concentration; thus, the harvest was conducted for the bud to mid-flower stages of cup-plant or alfalfa. The harvests were on June 20 and July 15 for cup-plant and on May 23 and July 24 for alfalfa. The harvested alfalfa and cup-plant were dried at 60℃ in a dryer for 72 hours. Samples were ground with a Wiley mill to pass through a 1-mm screen and stored in glass jars.

Chemical composition of forages

The content of ADF was determined by the sequential analysis method described by Hintz et al. (1996), which was a modification of Robertson and Van Soest (1977). The sample size was 0.5 g and 0.1 ml α-amylase (Sigma Chemical Co., St. Louis, Mo. number A 5426) was added during refluxing in the neutral detergent solution and again during sample filtration. The content of ADL (acid detergent lignin) was determined by sequential analysis (Goering and Van Soest, 1970), and crude protein concentration was determined by the Leco FP-528 system.

Determination of model selection

The model used to describe the digestion of cell wall components was a first order kinetic model (Mertens and Loften, 1980).

$$C=Cd \exp.-k\ (t-L)+Ci$$

C : concentration of constituent remaining after t hours of fermentation when t>L and C=Cd+Ci when t<L.
Cd : potentially digestible constituent concentration
k : rate constant
L : discrete lag time
Ci : indigestible constituent concentration.

Collection and preparation of innoculum

Rumen fluid was collected in a pre-warmed thermos. After the one-half solid on top of fluid layer was discarded. The content was poured into a waring blender under vacuum. The blending was done for 60 sec. The rumen fluid was squeezed through layers of cheese clothes and filtered through a mesh screen.

Incubation and analysis of cell wall residue

In vitro incubation followed Mertens and Loften (1980), which is a modification of the Goering and Van Soest method (1970). About 0.5 g of forage sample was incubated for 0, 3, 6, 9, 14, 20 28, 36, 48, and 72 h. Each incubation time was duplicated except the 48 h incubation. After each incubation time, neutral detergent fiber residues in samples were recovered without use of sodium sulfite. Dry matter content in samples was determined with 2.0 g of plant material which was dried at 105℃ for 24 h. Neutral detergent fiber at time 0 (RES0) was determined with the samples incubated at zero time. The extent of NDF digestion was calculated as the percentage of NDF digested at 96 h, and the digestion rate constant was determined by the slope of the line obtained by regression.

Statistical analysis

Data was analyzed by PROC GLM of the SAS Institute (1988). The experimental design was randomized complete block design with a split plot arrangement replicated four times. The forage species were main plot, and maturity was subplot treatments within the two locations. Statistical significance was determined using analysis of variance procedures and F tests.

The general linear model is Y=Species+Maturity +Location+(Species×RES0 level)+error. The direct non-linear square method from Mertens and Loften (1980) was tried to determine the parameters.

RESULTS AND DISCUSSION

The chemical composition of the plant materials used in these experiments are presented in table 1. The ADF contents of alfalfa and cup-plant from Lancaster were higher than those from Arlington. The forage quality was relatively more uniform in alfalfa. The ADL content in cup-plant was 57 to 82% of that in alfalfa. However, mean of ash content in cup-plant was about 42.7% higher than that in alfalfa.

The coefficient correlation and residual analysis indicated that DNLS was a reasonable method to fit the kinetic model. Table 2 shows the lag time, digestion rate, and potential extent of digestibility of alfalfa and cup-plant determined by DNLS method. Alfalfa had a shorter lag time than cup-plant ($p<0.05$). Mertens and Loften (1980) explained the difference of lag time among the forages with the inherent chemical or physical alteration and extensive hydration before the attachment of enzyme to fiber and activation of enzymes. From the shorter lag time, alfalfa seemed to have potential for more rapid digestion in rumen. However, the comparison of digestion rate showed no significant differences. Digestion rate of cup-plant in

Table 1. Chemical composition of alfalfa and cup-plant from Arlington and Lancaster, WI

Forage	Location	Maturity	Ash	ADF	ADL	IVDDM
			g/kg DM			
Alfalfa	Arlington	B[1]	57.3	280.5	71.6	636
		MF	65.3	325.8	81.0	592
	Lancaster	B	65.2	304.3	72.9	607
		MF	70.1	325.7	79.5	588
Cup-plant	Arlington	B	113.8	294.2	40.9	697
		MF	100.3	318.8	50.5	633
	Lancaster	B	113.5	320.0	43.9	723
		MF	103.7	375.4	65.2	522

[1] B=bud; MF=mid-flower.

Table 2. Effect of forage type and maturity on lag time, digestion rate, and potential digestibility of alfalfa and cup-plant

Forage	Location	Maturity	Direct nonlinear least square method			
			RESO[2] g/kg DM	Lag time hr	Dig. rate hr^{-1}	PD[3] g/kg NDF
Alfalfa	Arlington	B[1]	471	0.52	0.115	412
		MF	492	0.67	0.095	405
	Lancaster	B	461	1.08	0.083	395
		MF	460	1.54	0.089	396
Cup-plant	Arlington	B	427	1.61	0.102	436
		MF	459	2.31	0.082	390
	Lancaster	B	486	1.32	0.072	459
		MF	552	2.20	0.110	322
RMSE[4]				0.250	0.252	0.446
Probability of significance of effect						
Species			0.45	0.05	0.72	0.84
Location			0.04	0.56	0.38	0.81
RESO level			0.14	0.59	0.50	0.07

[1] B=bud; MF=mid-flower. [2] RESO=NDF residue at 0 time. [3] PD=potential digestibility. [4] RMSE=root mean square error.

Lancaster was not matched to the classical relationship between maturity and digestion rate. Potential extent of digestibility was not significantly different by species and location.

The amount of the indigestible fraction in NDF was not significantly different in either forage. However, cup-plant showed a higher indigestible portion than a alfalfa at mid-flower stage in both locations. Within the small range of NDF concentration, alfalfa showed less variation in indigestible fraction. The proportion of the indigestible fraction in NDF showed that the increase of NDF content in forage significantly related to the increase of indigestible fraction (table 3). Mertens and Loften (1980) considered the ratio of indigestible fiber portion to lignin content as a criterion for potential digestibility. The impact of lignin content on indigestible fraction was about four unit higher in cup-plant than in alfalfa because ADL content in cup-plant samples was about half of those in alfalfa samples although the indigestible fractions were similar in both forages. The range of the ratios of indigestible fraction to ADL content was wider in cup-plant samples than in alfalfa. The impact of ADL on indigestible fraction was small in matured cup-plant although the ADL content increased in matured cup-plant. This means the increased rate of indigestible fraction in NDF does not follow the steep increase of lignin concentration after the mid-flower stage in both locations. The slower increase of indigestible fraction probably affects the slow decline of *in vitro* digestibility of cup-plant. Among the digestion kinetics parameters in the DNLS method, indigestible fraction is more important in explaining the different digestion kinetics between the alfalfa and the cup-plant samples.

The differences between estimated dry matter digestibility (table 3) calculated by Goering and Van Soest (1970) and *in vitro* dry matter digestibility (table

Table 3. Effect of NDF concentration on the indigestible fraction in alfalfa and cup-plant from the two locations

Forage	Location	Maturity	Direct nonlinear least square method			
			RESO[2] g/kg DM	Indig.[3] fraction g/kg NDF	EST[4] DDM g/kg DM	Indig.[5] fraction / ADL
Alfalfa	Arlington	B[1]	471	588	555	8.2
		MF	492	596	532	6.6
	Lancaster	B	461	605	546	7.5
		MF	460	602	544	7.6
Cup-plant	Arlington	B	427	564	585	14.3
		MF	459	610	544	10.8
	Lancaster	B	486	541	546	12.4
		MF	552	678	457	10.4
Probability of significance of effect						
Species			0.45	0.50	0.28	0.01
Location			0.04	0.04	0.01	0.82
RESO level			0.14	0.01	0.01	0.04

[1] B=bud; MF=mid-flower. [2] RESO=NDF redidue at 0 time. [3] Indigestible fraction.
[4] ESTDDM, estimated digestible dry matter (Goering & Van Soest, 1970); DMD=(100-NDF)×0.98+NDF digestibility×NDF-12.9.
[5] Indigestible fraction/ADL.

1) were ranged from 44 to 81 g/kg DM in alfalfa, and 65 to 177 g/kg DM in cup-plant. The difference between ESTDDM (estimated digestible dry matter) and IVDDM (*in vitro* digestible dry matter) decreased with the progress of maturity in both forages. This discrepancy showed a similar trend with the ratio of the indigestible fraction to ADL. However, underestimation of digestibility by ESTDDM could not reflect fully the digestion characteristics of non-uniform NDF in cup-plant.

CONCLUSION

Cup-plant has similar digestion parameters to alfalfa. However, the shorter lag time of alfalfa probably causes rapid digestion. However, relatively slowly increasing indigestible fraction and low amount of lignin make *in vitro* digestibility of cup-plant less changeable by maturation than that of alfalfa. To understand more about the digestion kinetics in cup-plant, soluble nutrient portion relating NDF digestion needs to be studied in the future.

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