Predicting In Vivo Dry Matter, Energy, and Protein Digestibility of Deer Forages

Thomas G. Barnes, *Forestry Department, University of Kentucky, Lexington, KY 40546–0073*

Larry W. Varner, *No. 11 Donna Drive, Uvalde, TX 78801*

Lytle H. Blankenship, *Box 5220, Uvalde, TX 78802*

James F. Gallagher, *Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843*

Abstract: We investigated 4 in vitro digestion procedures to estimate dry matter, energy, and apparent protein digestibilities of 2 southern Texas white-tailed deer (*Odocoileus virginianus*) forages. Standard 2-stage rumen inoculum technique consistently underestimated in vivo dry matter digestibility by 1% to 9%, but could be corrected to in vivo values by regression analyses (R^2 = 0.89). This technique also predicted digestible energy (kcal/g) and digestible protein (g/100g feed) accurately (R^2 = 0.84 and 0.71, respectively). Cellulase, pepsin, or multienzyme techniques did not predict dry matter, energy, or protein digestibility accurately or consistently

Proc. Annu. Conf. Southeast Assoc. Fish and Wildl. Agencies 43:297–305

Nutrient quality and availability are the major parameters affecting carrying capacity of deer habitat. The nutritional quality of deer forages, based on chemical analyses, often is overestimated. The overestimation of browse nutrient quality may be attributed to the presence of condensed tannins, which reduce dry matter and protein digestibilities (Robbins et al. 1987*a, b;* Barnes 1988). Thus, digestible nutrients, not gross energy or crude protein (CP), are usually the important variables in meeting animal nutrient requirements. Determination of digestible nutrients has been done traditionally using in vivo digestion trials which are expensive, time consuming, and labor intensive.

The development of the 2-stage rumen inoculum-pepsin digestion (Tilley and Terry 1963) technique greatly expanded opportunities for evaluating forage quality. The primary disadvantage is related to the cost of maintaining rumen-fistulated animals. Consequently, several enzymatic techniques have been developed to predict

dry matter (Dowman and Collins 1982, Hadjipanayiotou et al. 1987, Valdes and Jones 1987) and protein digestibilities (Wohlt et al. 1973, Hsu et al. 1977). These techniques were developed and evaluated using grass, legume, or concentrate diets and have not been evaluated for use in estimating digestibilities of forages containing tannins.

Robbins et al. (1987*b*) suggested summative equations could be used to estimate digestibility parameters in browsing animals because differences in cell wall digestion due to tannins complicates in vitro techniques. Therefore, 2-stage rumen inoculum techniques could be used as a valid model for sheep, cattle, or other grazers, but not for browsers such as deer. Our objective was to compare rumen inoculum and enzymatic digestion techniques to determine their applicability for estimating in vivo nutrient digestibilities of 2 deer forages in southern Texas.

We acknowledge financial support from the Caesar Kleberg Program in Wildlife Ecology at Texas A&M University and the National Rifle Association. The assistance of T. J. Fillinger, G. M. Wampler, C. M. Hensarling, and T. J. VanNorman is appreciated. N. J. Silvy, W. E. Grant, and anonymous reviewers provided helpful comments on earlier drafts of this manuscript. This is contribution 24131, Texas Agricultural Experiment Station.

Methods

Forage species studied were selected based on published studies (Varner and Blankenship 1987) and examination of rumen contents of sacrificed deer from Dimmit, LaSalle, Maverick, Uvalde, and Zavala counties, Texas (Varner unpub. data). Guajillo (*Acacia berlandieri*) was examined seasonally because it is 1 of the dominant forages consumed during all seasons by deer and cattle. Brazil (*Condalia obovata*) was used because, among shrubs, it has a high dry matter digestibility (DMD) (Varner et al. 1977) when it occurs commonly in deer diets. We also fed a pelleted ration (Barnes 1988) because it did not contain tannins and is used often by ranchers as a supplemented ration in southern Texas.

We conducted 6 completely randomized balance trials using browse during the period June 1986 to July 1987. We also conducted 3 completely randomized balance trials using a pelleted ration during the winter, spring, and summer 1986.

Browse stems were collected in the field. Guajillo leaves were stripped by hand and fed fresh or stored overnight under refrigeration and fed the following morning. Brazil leaves were stripped mechanically (Gallagher et al. 1988), frozen, and fed immediately upon thawing. It is important to feed fresh forages because handling affects the quantity of phenolic compounds in the vegetation (Servello et al. 1987).

Deer ($N = 6$) were confined in individual metabolism crates housed in a climate-controlled room. Temperatures closely approximated outdoor temperatures during all seasons except summer when daytime temperatures never exceeded 27° C. Animals were weighed prior to each trial, which consisted of a 5-day adjustment period followed by a 5-day total fecal collection period. Seven day adjustment

periods are recommended for reduction of variance (Mothershead et al 1972), but we used a 5-day period to reduce stress and maintain maximum intakes. To ensure an adequate microbial fauna capable of digesting browse, animals were supplemented prior to each trial with browse. Forage was offered ad libitum once in the morning during the pretrial period, reduced until no orts remained, and fed at this level during the collection period. Water was available ad libitum.

Forage, orts, and fecal samples were taken daily, oven-dried at 60° C, ground through a 1-mm screen in a Wiley Mill, composited for the total collection period, and subsampled for analyses.

Gross energy of feed and feces were determined using a Parr adiabatic oxygen bomb calorimeter. Feed and fecal samples were wet digested using perchloric acid and hydrogen peroxide (Adler and Wilcox 1985). Crude protein (CP) was determined colorimetrically on wet-digested samples (Laubner 1975). Condensed tannins were extracted in methanol and measured colorimetrically using a catechin standard (Burns 1971).

Digestible energy (kcal/g) was calculated by multiplying gross energy × apparent digestibility. Digestible protein (g/100 g feed) was calculated by multiplying CP × apparent digestibility (Robbins et al. 1987*a*). No estimate of metabolic fecal nitrogen could be obtained because a Lucas test (Van Soest 1982) is invalid for diets containing tannins (Robbins et al. 1987a). True protein digestibility of the pelleted ration was calculated using a metabolic fecal nitrogen value of 4.88 g/100 g feed (Robbins et al. 1974) and removed from digestibility equations.

In vitro DMD was estimated using 2 techniques: (1) 2-stage rumen fermentation using inoculum from a fistulated steer grazed on native range (Tilley and Terry 1963, modified by Newman 1972), and (2) a pepsin/cellulase digestion (0.1 or 0.75 N pepsin/HCL solution in 1 liter of 0.05M sodium acetate buffer adjusted to pH 4.6, Hadjipanayiotou et al. 1987). Samples were run in triplicate with 1 replication.

Protein digestibilities were predicted using 2 enzymatic digestions: (1) pepsin/HCL digestion using a 0.1N pepsin/HCL solution outlined in the second stage of the Tilley and Terry (1963) technique; and (2) multienzyme technique using a reduction in pH after 1, 5, and 10 minutes (Hsu et al. 1977). Samples for both protein prediction techniques were run in triplicate with 1 replication.

Apparent digestibility was calculated as:

$$\frac{\text{feed intake} - \text{fecal output}}{\text{feed intake}}$$

One-way ANOVA and least significant differences mean comparison tests were used to detect significant differences ($P \leq 0.05$) in nutrient digestibilities. In vitro nutrient digestibilities were corrected to in vivo values using simple linear regression. Pelleted diet results were not included in the regression equations because this diet did not contain tannins and was not reflective of natural diets. Additionally, use of these results would have biased the data limiting equation predictive value for natural deer forages.

Results and Discussion

Gross energies and ash content of browse were similar but CP and condensed tannins varied (Table 1). Chemical composition of the pelleted diet was different from browse (Table 1). Mean deer weight was 39.4 kg and did not differ ($P = 0.10$) between trials (Table 2). Mean dry matter intake (DMI) was 16.1 g/kg body weight/day and differed between trials (Table 2). There were no seasonal differences in nutrient digestibilities using the pelleted diet. Consequently, data were pooled and tested for differences attributed to sex and age. There were no intake differences attributed to sex; however, yearling deer had higher ($P \leq 0.01$) DMI than adults (Table 2). The intake difference observed between yearling and adult deer was expected because nutrient digestibilities were not different; therefore, young deer meet higher nutrient requirements through increased intake. Differences in seasonal intake of browse also were expected because of a range of behavioral, morphological, and physiological mechanisms (Allison 1985).

In vivo DMD averaged 42.2% and differed between trials (Table 2). The 2-stage in vitro DMD (Table 3) was corrected to in vivo DMD ($Y = -21.8 + 1.7X$, $R^2 = 0.89$, $P = 0.005$) and was valid for predicting in vivo DMD for browsing animals. The Tilley and Terry (1963) 2-stage rumen fermentation technique predicted in vivo DMD accurately within the range of our data set (35%–49% digestible). Over 50% of southern Texas shrubs known to occur in deer diets have DMD values within this range (Varner et al. 1977). Lower in vitro DMD values (1%–9% below in vivo DMD) can result from different inoculum scores (Blankenship et al. 1982, Priebe et al. 1987), diets of donor animals (Campa et al. 1984), or insufficient inoculum samples (Clary et al. 1988). However, these values can easily be corrected to in vivo estimates by regression analysis. Standard or index forages should be used to make in vitro DMD results more consistent among studies to predict more accurately the actual digestibility of the forage.

Digestible energy (Table 2) was highly correlated with in vivo DMD (Y =

Table 1. Chemical composition of forage fed to white-tailed deer in digestion studies.

Diet and Season	Crude Protein (%)	Gross Energy (kcal/g)	Condensed Tannins (mg/g)	Ash (%)
Guajillo				
Spring	20.0	4.74	92.1	5.0
Early Summer	20.2	4.71	89.9	6.4
Late Summer	15.6	4.72	112.8	7.3
Fall	16.8	4.69	108.1	7.0
Winter	17.6	4.78	117.9	5.7
Brazil				
Spring	13.6	4.19	64.6	11.6
Pelleted	22.5	3.83	0	14.5

Table 2. Body weight (BW); dry matter intake (DMI); apparent in vivo dry matter (DMD), energy, and protein digestibility; and digestible energy and protein of forage fed to white-tailed deer in digestion studies.

Diet and Season	N	BW (Kg) $\bar{x}$	BW (Kg) SE	DMI (g/kg BW/day) $\bar{x}$	DMI (g/kg BW/day) SE	DMD (%) $\bar{x}$	DMD (%) SE	Apparent Protein Digestibility (%) $\bar{x}$	Apparent Protein Digestibility (%) SE	Apparent Energy Digestibility (%) $\bar{x}$	Apparent Energy Digestibility (%) SE	Digestible Energy (kcal/g) $\bar{x}$	Digestible Energy (kcal/g) SE	Digestible Protein (g/100g feed) $\bar{x}$	Digestible Protein (g/100g feed) SE
Guajillo															
Spring	5	43.7	5.2	11.7	1.4	48.1	0.8	45.8	2.2	46.0	0.9	2.18	0.02	9.14	0.20
Early Summer	5	49.3	6.4	13.0	1.9	41.2	2.7	35.5	4.1	39.1	2.8	1.85	0.05	7.31	0.33
Late Summer	5	30.5	2.4	19.6	2.5	35.2	2.4	13.7	3.5	32.5	2.7	1.53	0.05	2.14	0.22
Fall	6	36.8	2.4	13.3	1.8	38.3	2.3	20.5	5.2	35.9	2.3	1.68	0.04	3.51	0.37
Winter	5	41.1	5.0	20.0	1.5	41.5	0.6	22.7	2.5	38.6	0.7	1.84	0.01	4.01	0.21
Brazil															
Spring	5	38.2	1.0	19.4	1.4	49.1	2.1	38.1	4.5	45.5	2.3	1.91	0.04	5.20	0.26
Pelleted	11	37.0	1.0	21.0A[a]	0.6	75.6	0.9	95.0[b]	0.04	77.6	1.0	2.96	0.01	16.38	0.10
				32.4Y	1.3										

[a]Significant ($P \leq 0.01$) difference in DMI/kg BW/day between adult (A) ($N = 7$) and yearling (Y) ($N = 4$) deer.
[b]True protein digestibility based on 4.88 g/100 g feed metabolic fecal nitrogen (Robbins et al. 1975) which was removed from digestibility equations.

Table 3. Two-stage in vitro and cellulase dry matter digestibility (DMD), and pepsin/HCL and multienzyme protein digestion of forages fed to white-tailed deer in digestion studies.

Diet and	2-stage in vitro DMD (%)		Cellulase DMD 0.1N HCL (%)		Cellulase DMD 0.75N HCL (%)		Pepsin/HCL Protein digestion (%)		Multienzyme protein digestion (pH reduction)					
									1 minute		5 minutes		10 minutes	
Season	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Guajillo														
Spring	41.5	0.68	23.8	0.96	18.6	1.21	42.4	0.23	0.09	0.009	0.13	0.006	0.16	0.007
Early Summer	37.3	0.37	20.8	1.12	27.8	1.74	37.7	0.41	0.11	0.006	0.13	0.007	0.21	0.016
Late Summer	34.2	0.13	31.6	2.73	27.3	1.78	36.9	0.24	0.06	0.004	0.10	0.003	0.16	0.003
Fall	34.5	0.13	27.8	0.42	32.3	2.41	38.3	0.11	0.09	0.008	0.14	0.007	0.24	0.003
Winter	35.5	0.25	22.4	1.24	19.2	0.61	36.9	0.14	0.11	0.009	0.16	0.006	0.26	0.013
Brazil														
Spring	40.0	0.32	47.0	1.57	38.3	1.10	31.4	0.28	0.12	0.006	0.18	0.005	0.23	0.003

$-6.9 + 0.9X$, $R^2 = 0.99$, $P = 0.001$) and 2-stage in vitro DMD (Y $= -23.9 + 1.7X$, $R^2 = 0.92$, $P = 0.002$). Digestible energy (kcal/g) could be predicted using 2-stage in vitro DMD values (Y $= -0.67 + 0.07X$, $R^2 = 0.84$, $P = 0.01$). The close relationship between in vivo DMD and digestible energy has been reported previously (Robbins et al. 1975), and allows managers to predict digestible energy using 2-stage in vitro DMD values.

Apparent protein digestibility (Table 2) was highly correlated to in vivo DMD (Y $= -56.1 + 2.0X$, $R^2 = 0.81$, $P = 0.01$). Apparent protein digestibility could be predicted using 2-stage in vitro DMD values (Y $= -118.0 + 4.0X$, $R^2 = 0.92$, $P = 0.002$). Digestible protein (Table 2) could be predicted using 2-stage in vitro DMD values (Y $= -21.7 + 0.7X$, $R^2 = 0.71$, $P = 0.03$). Our data show the 2-stage rumen fermentation technique can be used to predict digestible protein, which provides managers an effective laboratory method for determining stocking rates and carrying capacity based on levels of digestible protein in forage rather than CP.

In vitro cellulase DMD using 0.1 N HCL (Table 3) was not correlated to in vivo DMD (Y $= 33.7 + 0.3X$, $R^2 = 0.29$, $P = 0.29$). In vitro cellulase DMD (Table 3) using 0.75 N HCL was not correlated to in vivo DMD (Y $= 39.5 + 0.01X$, $R^2 = 0.02$, $P = 0.79$). Previous experiments using fungal cellulases succeeded in predicting in vivo DMD of grasses, legumes, and concentrate diets (Dowman and Collins 1982, Valdes and Jones 1987). These diets contain low levels of condensed tannins, which reduce DMD (Robbins et al. 1987*b*, Barnes 1988) and are known to inhibit cellulase activity (Borneman et al. 1986, Varel and Jung 1986). The failure of the cellulase technique to accurately predict in vivo DMD in our study may be related to the presence of condensed tannins. Cellulase may not be an appropriate enzyme to use because deer rumen contain few celluolytic bacteria (Kay et al. 1980) and digest little cellulose in vivo (Barnes 1988).

The in vitro enzymatic protein digestion procedures did not predict in vivo apparent protein digestibility. Pepsin/HCL protein digestibility was not correlated with in vivo apparent protein digestibility (Y $= 41.9 + 0.05X$, $R^2 = 0.09$, $P = 0.95$). Reducing pH after 1, 5, or 10 minutes was not correlated with apparent protein digestibility (Y $= -5.41 + 366.3X$, $R^2 = 0.41$, $P = 0.17$; Y $= 6.65 + 162.4X$, $R^2 = 0.13$, $P = 0.49$; and Y $= 0.23 - 0.0006X$, $R^2 = 0.03$, $P = 0.50$ for 1, 5, or 10 minutes, respectively). In vitro enzymatic methods for determining protein digestibility have not been accepted widely because procedures are complicated or results are not compared with corresponding in vivo data. The technique developed by Hsu et al. (1977) accurately predicted protein digestibility of the pelleted diet (85.4% in vitro vs. 95.0% true in vivo protein digestibility). However, the technique did not accurately predict digestible protein of browse, possibly because of the presence of condensed tannins (Robbins et al. 1987*a*, Barnes 1988).

We recommend, for the limited range of data presented, the 2-stage rumen fermentation technique as a fast, inexpensive, and reliable method of predicting DMD, digestible energy, and digestible protein of deer forages. Further work expanding the range of digestible energy and protein predictive capabilities using

this technique is needed. Also, studies need to be conducted to develop a reliable enzymatic technique for predicting those same parameters.

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