Variation in Ruminant Preference for Alfalfa Hays Cut at Sunup and Sundown[1]

Dwight S. Fisher,* Henry F. Mayland, and Joseph C. Burns

ABSTRACT

Diurnal variation in the concentration of total nonstructural carbohydrates (TNC) occurs in plants as a result of photosynthesis. Ruminants have been shown to prefer tall fescue (*Festuca arundinacea* Schreber) hays cut in the afternoon but the effect of morning vs. evening cutting had not been tested in legumes. To test for diurnal variation in preference for alfalfa (*Medicago sativa* L.), we harvested six times in the midbud stage. Harvests were paired so that each time a cutting of alfalfa was made at sundown (PM) another was made the next morning at sunup (AM). We harvested in this manner three times resulting in six hays. The hays were field dried, baled, and chopped prior to their use 3 to 6 mo after harvest. Three experiments were conducted [Exp. 1, sheep (*Ovis aries*); Exp. 2, goats (*Capra hircus hircus*); and Exp. 3, cattle (*Bos taurus*)] utilizing six animals in each case. During an adaptation phase, hays were offered alone as meals. In the experimental phase, every possible pair of hays (15 pairs) was presented for a meal. Data were analyzed by multidimensional scaling as well as by traditional analyses. Multidimensional scaling indicated that the animals were basing selection on at least two criteria. Variables associated with preference through multiple regression varied across experiments but significant coefficients were found between preference and nitrate, protein, carbohydrate fractions, lignin, and cellulose. Coefficients varied depending on which other variables were in the model; however, carbohydrates were associated with positive coefficients. Shifting hay mowing from early in the day to late in the day was effective in increasing forage preference as expressed by short-term dry matter intake.

THE CONCENTRATION OF TNC in forage plants increases as photosynthate accumulates because carbon export does not keep pace with carbon fixation during the photoperiod. Concentrations of nonstructural carbohydrates have been observed to be higher in the afternoon than at dawn (Allen et al., 1961; Lechtenberg et al., 1971). Diurnal patterns of forage intake rates in sheep have been observed to coincide with increases in nonstructural carbohydrates (Orr et al., 1997). If ruminants generally prefer forages with higher nonstructural carbohydrate, then preference for hays cut within the same 24-h period may vary after a period of light or darkness. Food preferences are affected by many factors (Forbes and Kyriazakis, 1995; Early and Provenza, 1998) and diurnal variation in nonstructural carbohydrate results in only a subtle change in forage composition. In this case, forage composition is modified without the use of supplements and provides a rigorous test of the ruminant's ability to detect that one feed varies slightly from another (Provenza and Balph, 1987). To express a preference for hay cut at a particular time of day, the ruminant must be able to recognize the forage when it offered with other forages harvested from the same source. In the current study, we tested for variation in short-term preference as expressed in dry matter intake (DMI) for alfalfa (*Medicago sativa* L.) hays harvested from the same field approximately 9 to 11.5 h apart.

MATERIALS AND METHODS

Field Procedures

Hay was harvested from an established field of Germain WL322 HQ alfalfa (4 fall dormancy) near Kimberly, ID, six times in the midbud stage. The initial growth was cut and discarded. The subsequent cuts were paired so that each cutting at sundown (PM), after a sunny day, was followed by another cutting the next morning (AM) at sunup. We cut hays in this manner three times resulting in six hays (Hay 1, 8 July PM; Hay 2, 9 July AM; Hay 3, 13 August PM; Hay 4, 14 August AM; Hay 5, 22 September PM; and Hay 6, 23 September AM). The 1-h cutting interval straddled sundown or sunup. Weather during haymaking was relatively clear and there was no precipitation (Table 1). Daytime air temperature ranged from 31 to 22°C with nighttime lows ranging from 12 to 7°C. The hays were field dried and baled in square bales weighing approximately 60 kg prior to shipping to Raleigh, NC, for the preference trials. Hays cut at PM were ready for baling at approximately the same time as the hays cut at AM. The decision to bale was based on an estimate that the DM of the hay was over 850 g kg^{-1} by an individual with experience in haymaking. There were no significant differences in the DM contents of the hays when fed to the three animal species (data not shown) and the average DM was approximately 940 g kg^{-1}.

Alfalfa (neutral detergent fiber = 430 g kg^{-1}, crude protein = 210 g kg^{-1}, in vitro true dry matter disappearance = 750 g kg^{-1}) was harvested in the late vegetative stage as hay at Raleigh, NC and fed ad libitum to the sheep and goats each day after the animals had finished with the experimental forages. Cattle were fed switchgrass hay (neutral detergent fiber = 710 g kg^{-1}, crude protein = 120 g kg^{-1}, in vitro true dry matter disappearance = 780 g kg^{-1}) each day after the morning experimental phase. The switchgrass was harvested in the vegetative stage at Raleigh, NC. All hays were stored on pallets undercover in the same metal building. Just prior to feeding, and to minimize leaf loss, all hays were passed through a hydraulic Van Dale S600 Bale Processor (J. Star Industries, Ft. Atkinson, WI) with stationary knives spaced

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D.S. Fisher, USDA-ARS, 1420 Experiment Station Road, Watkinsville, GA, 30677; H.F. Mayland, USDA-ARS, Kimberly, ID 83341; and J.C. Burns, USDA-ARS, Dep. of Crop Science, and Dep. of Animal Science, NC State Univ., Raleigh, NC 27695. Received 16 Oct. 2000. *Corresponding author (dwight_fisher@scientist.com).

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Table 1. Climate data for days during which experimental hays were cut.

	Cut	Air temperature				Radiation	
		Maximum	Minimum	Sunrise	Sunset	Actual	Potential
		°C		MDT		MJ m^{-2} d^{-1}	%
Hay 1	**8 July, PM**	**31**	**11**	**0607**	**2116**	**29.9**	**93**
Hay 2	**9 July, AM**	**28**	**11**	**0608**	**2115**	**24.6**	**77**
Hay 3	**13 August, PM**	**28**	**9**	**0641**	**2041**	**25.7**	**92**
Hay 4	**14 August, AM**	**29**	**12**	**0642**	**2040**	**26.0**	**93**
Hay 5	**22 September, PM**	**22**	**8**	**0724**	**1934**	**19.0**	**89**
Hay 6	**23 September, AM**	**23**	**7**	**0725**	**1932**	**19.5**	**92**

10 cm apart. This resulted in a consistent cut with hay length ranging from 8 to 13 cm.

Design of Preference Trials

Three experiments were conducted that differed in the animal species used for determining preference. All experimental protocols involving animals were approved by the institutional animal care and use committee. In Exp. 1, six Katahdin ewe sheep were used (mean BW = 46 kg), in Exp. 2, six Spanish doe goats were used (mean BW = 54 kg), and in Exp. 3 six Hereford steer cattle were used (mean BW = 661 kg).

Prior to the experimental phase an adaptation or training period (Kyriazakis et al., 1990) was conducted in which a meal of each hay was offered to each animal to allow an association of the hay with any post-ingestive feedback produced by the forage. The order in which the hays were offered individually during the training period was randomized separately for each animal.

During the experimental phase, each possible pair of the six hays (15 pairs) was presented for a meal but only one pair was offered each day. The order of presentation of the pairs was randomized, as was the left-right position of the hays in the pair. The weight of hay was determined prior to, and after, feeding. This permitted calculation of dry matter consumed after adjusting for the dry matter concentration of the hay. When presented with a pair of forages, sheep and goats were offered approximately 0.75 kg of each hay and allowed approximately 2.5 h to feed. In Exp. 3, cattle were offered approximately 2 kg of each hay and allowed approximately 30 min to feed. In Exp. 3, time-lapse video was used during the 30-min feeding period to estimate time spent at each feeder in order to calculate intake rate during the preference trial. In all three experiments care was taken to provide a sufficient amount of each hay so that animals always had a choice between the two hays in the pair. As was discussed previously, each day after the preference trial the animals were fed ad libitum with a hay not included in preference trial.

Forage Nutritive Value

Forage samples were composited in each experiment from sub-samples collected at each feeding. Samples were composites representing the forage offered each animal for each hay ($n = 6$) in each experiment.

In vitro true dry matter disappearance (IVTDMD) was determined on hay samples with ruminal inoculum collected from a cannulated mature Hereford steer fed a mixed alfalfa and orchardgrass (*Dactylis glomerata* L.) hay. After batch incubation with ruminal inoculum combined with artificial saliva in fermentation vessels (Ankom Technology Corp., Fairport, NY) samples were extracted with neutral detergent solution for estimation of IVTDMD.

Fiber fractions were estimated (NDF, ADF, cellulose, sulfuric acid lignin, and ADIA) according to Van Soest and Robertson (1980) in a batch processor (Ankom Technology Corp., Fairport, NY). Crude protein was calculated as 6.25 times the percentage of N as determined with an autoanalyzer (AOAC, 1990).

The TNC were analyzed by an adaptation (Fisher and Burns, 1987) of the method described by Smith (1969). The TNC were fractionated into monosaccharides, disaccharides, short chain polysaccharides, and starch. Starch was determined by digesting to glucose with amyloglucosidase and reading the monomer concentration on a YSI Model 27 Industrial Analyzer (Yellow Springs Instrument Co., Yellow Springs, OH).

All samples were scanned using a near infrared reflectance (NIR) spectrophotometer. Laboratory determinations were used to estimate composition on samples selected on the basis of observed differences in NIR spectra. These laboratory observations were then used with the NIR spectra to develop prediction equations for the following variables listed with their means along with their standard errors of cross validation and the number of laboratory determinations in the calibration set; IVTDMD (727 g kg^{-1} ± 18 g kg^{-1}, $n = 162$), NDF (464 g kg^{-1} ± 8 g kg^{-1}, $n = 162$), ADF (350 g kg^{-1} ± 8 g kg^{-1}, $n = 162$), cellulose (266 g kg^{-1} ± 6 g kg^{-1}, $n = 162$), lignin (76 g kg^{-1} ± 2 g kg^{-1}, $n = 162$), ADIA (4 g kg^{-1} ± 2 g kg^{-1}, $n = 162$), crude protein (196 g kg^{-1} ± 5 g kg^{-1}, $n = 162$), monosaccharides (12 g kg^{-1} ± 1 g kg^{-1}, $n = 80$), disaccharides (17 g kg^{-1} ± 2 g kg^{-1}, $n = 80$), short chain polysaccharides (9 g kg^{-1} ± 2 g kg^{-1}, $n = 80$), starch (7 g kg^{-1} ± 2 g kg^{-1}, $n = 80$), and TNC (45 g kg^{-1} ± 4 g kg^{-1}, $n = 80$).

Nitrate Nitrogen (NO_3-N) concentrations were determined in the laboratory by auto analyzer using hydrazine reduction (Kamphake et al., 1967). A 200-mg sample was extracted with 50 mL of water, shaken for 30 min, filtered, and the extract analyzed for N and reported as NO_3-N.

Statistical Analysis

Variables describing the composition of the hays were tested by analysis of variance of samples collected and composited during the feeding of each animal. This allowed the error variance to include variation present in the feeding of each animal. Within a hay treatment, we did not remove variation by subsampling and mixing forage samples across animals. We subsampled the hays to estimate the mean composition of the diet fed to each animal in a given experiment. The model statement in the analysis of variance included animal and hay as the only effects.

By offering pairs we were able to use multidimensional scaling (Buntinx et al., 1997) as well as traditional statistical analyses. For multidimensional scaling (MDS), the difference between the pair of hays was expressed by subtracting the amount of the least preferred hay from the most preferred hay and dividing by the sum of the two intakes. In this way, preference was expressed as a difference ratio. If the animal consumed equal quantities of the hays in the pair, then the difference ratio was equal to zero and no preference was expressed between the two hays. If only one of the pair was consumed, then the difference ratio was equal to one and the

Table 2. Short-term Intake of alfalfa hays fed in Exp. 1 (sheep), Exp. 2 (goats), and short-term intake and intake rates in Exp. 3 (cattle).

	Sheep	Goats	Cattle		
Forage	DMI†	DMI	DMI	DMIR	Time
	g			g min^{-1}	min
Hay 1‡	443	414	1022	108	9.9
Hay 2	415	385	842	101	8.8
Hay 3	444	456	619	100	5.6
Hay 4	341	298	324	140	3.2
Hay 5	473	494	1320	115	11.4
Hay 6	399	350	1107	111	9.9
MSD	63	62	240	42	2.2
Mean PM	453	455	987	108	9.0
Mean AM	385	344	758	117	7.3
PM vs. AM($P > F$)	<0.01	<0.01	<0.01	0.33	0.02

† Values are means of six animals; DMI = dry matter intake; DMIR = dry matter intake rate; Time = time spent at the feeder.

‡ Hays 1, 3, and 5 are PM cuts while Hays 2, 4, and 6 are AM cuts.

hays were judged to be most different (Buntinx et al., 1997). In MDS, the differences are then represented as points in a one or more dimensional space. The Euclidean distances between points are then calculated and compared with the observed *differences*. The location of the points in the one or more dimensional space are adjusted iteratively and a least-squares solution arrived at that approximates the observed differences with the Euclidian distances among points. In this way, we can estimate both the number of criteria (dimensions) being used determining preference as well as the relative magnitude of the preference (distance) among the hays.

Experimental effects were also tested by analysis of variance after averaging the DMI of hays across all pairs (n = 5) by each animal. In this approach, the analysis of variance included terms for animal and hay. Means were separated with the Waller-Duncan K-ratio t-test (K-ratio = 100) providing a minimum significant difference (MSD). An orthogonal contrast was used to test for the sundown (PM) vs. the sunrise (AM) cutting effect on DMI.

Simple linear correlation and stepwise regression were used to examine relationships between measures of nutritive value and preference as expressed in the MDS dimensions and in DMI relative to the other hays. During stepwise regression a significance level of 0.15 was used for selection and removal from the regression model.

RESULTS AND DISCUSSION

Short Term Intake

As was found with tall fescue (Fisher et al., 1999), all three animal species consumed more of the PM cut hays as compared with the AM hays cut within the same 12 to 15 h ($P < 0.01$) (Table 2). Sheep did not express a significant preference among the 3 PM cuts of hay but did prefer Hay 2 (AM cut) to Hay 4 (AM cut) (MSD = 63 g). Goats preferred Hay 5 (PM cut) to Hay 1 (PM cut) among the evening harvests and, like the sheep, preferred Hay 2 (AM cut) to Hay 4 (AM cut) (MSD = 62 g) among the morning harvests. Cattle expressed a number of significant preferences with Hay 5 (PM cut) preferred to Hay 1 (PM cut), which in turn was preferred over Hay 3 (PM cut) (MSD = 240 g). This was consistent with their preferences for the AM cut hays with Hay 6 the most preferred followed by Hay 2 and with Hay 4 the least preferred. Use of time-lapse video made it possible to calculate the dry matter intake rate (DMIR) for the cattle and this showed that there was no difference in DMIR but that the cattle spent more time (P = 0.02) at the feeders with PM hays.

Forage Composition

Estimates of forage composition were affected by cut and various effects were noted in the three experiments (Tables 3, 4, and 5). The significant effects on composition varied among the three experiments and are attributed to bale-to-bale variation during the course of the experiment combined with sampling and laboratory error.

In the case of Exp. 1 utilizing sheep (Table 3), significant effects were found because of PM vs. AM cuts on all fiber fractions, IVTDMD, and carbohydrate fractions. Crude protein was not affected by cutting time but NO_3-N was lower in the PM cut hays. Estimates of the fiber fractions (NDF, ADF, cellulose, hemicellulose, lignin, and ADIA) were lower in the PM cut hays. The carbohydrate fractions and digestibility (as estimated by IVTDMD) were higher in the PM-cut hays. Changes in assays such as the fiber fractions may be due to dilution by increased carbohydrates in the PM-cut hays.

In Exp. 2, which utilized goats, the only fiber component that was significantly decreased in the PM cut hays was hemicellulose (Table 4). Digestibility was higher in the PM cut hays and all of the carbohydrate fractions were higher in the PM hays relative to AM hays at the same cutting. Crude protein and NO_3-N were not different in this experiment.

The composition of hays in Exp. 3 (cattle) (Table 5)

Table 3. Composition of alfalfa hays fed to sheep in Exp. 1.

Forage	NDF†	ADF	Cell	Hemi	Lignin	ADIA	IVTDMD	CP	NO_3-N	MSAC	DSAC	SCPS	Starch	TNC
	g kg^{-1} DM													
Hay 1‡	411	309	237	102	66	3.6	766	220	0.54	12.6	15.1	8.9	8.5	45.1
Hay 2	439	335	256	104	72	4.2	743	210	0.63	9.6	13.4	8.5	8.6	40.1
Hay 3	406	312	240	94	66	4.3	774	226	0.94	14.0	19.8	9.4	9.1	52.3
Hay 4	433	329	252	104	72	4.6	746	224	1.08	9.4	13.3	8.5	7.8	39.0
Hay 5	380	291	223	89	60	4.9	805	228	0.76	16.8	32.9	11.3	10.4	71.4
Hay 6	382	295	225	88	62	5.7	792	226	0.81	12.4	28.5	10.9	7.5	59.3
MSD	22	19	15	6	4	0.5	15	16	0.11	1.1	2.4	0.2	1.0	3.3
Mean PM	399	304	233	95	64	4.3	782	225	0.75	14.5	22.6	10.0	9.3	56.2
Mean AM	418	320	244	99	69	4.8	760	220	0.84	10.4	18.4	9.3	8.0	46.1
PM vs. AM ($P > F$)	<0.01	<0.01	0.01	0.03	<0.01	<0.01	<0.01	0.20	0.01	<0.01	<0.01	<0.01	<0.01	0.01

† Means of six subsamples; NDF = neutral detergent fiber; ADF = acid detergent fiber; Cell = cellulose; Hemi = hemicellulose; Lignin = sulfuric acid lignin; ADIA = acid detergent insoluble ash; IVTDMD = in vitro true dry matter disappearance; CP = crude protein; NO_3-N = nitrate nitrogen; MSAC = monosaccharides; DSAC = disaccharides; SCPS = short chain polysaccharides; TNC = total nonstructural carbohydrates; MSD = minimum significant difference.

‡ Hays 1, 3, and 5 are PM cuts while Hays 2, 4, and 6 are AM cuts.

Table 4. Composition of alfalfa hays fed to goats in Exp. 2.

Forage	NDF†	ADF	Cell	Hemi	Lignin	ADIA	IVTDMD	CP	NO_3-N	MSAC	DSAC	SCPS	Starch	TNC
	g kg^{-1} DM													
Hay 1	398	303	235	95	65	3.8	771	226	0.54	13.5	14.6	8.7	6.7	43.5
Hay 2	425	322	249	103	69	4.3	750	219	0.56	11.4	11.3	8.0	5.9	36.6
Hay 3	411	314	243	97	67	4.7	766	227	0.97	13.8	18.2	9.0	7.2	48.2
Hay 4	415	317	247	98	68	4.2	755	230	1.01	10.5	11.5	8.3	5.2	35.6
Hay 5	373	288	222	86	60	5.5	806	230	0.76	17.7	32.9	11.0	7.9	69.5
Hay 6	372	285	220	87	60	5.9	794	232	0.76	13.0	26.1	10.6	5.4	55.1
MSD	20	17	13	5	4	0.6	15	ns	0.07	0.9	2.2	0.3	1.7	3.2
Mean PM	394	302	233	93	64	4.7	781	227	0.76	15.0	21.9	9.6	7.3	53.7
Mean AM	404	308	239	96	66	4.8	766	227	0.78	11.6	16.3	8.9	5.5	42.4
PM vs. AM ($P > F$)	0.10	0.18	0.18	0.02	0.15	0.41	<0.01	0.89	0.42	<0.01	<0.01	<0.01	<0.01	<0.01

† Means of six subsamples; NDF = neutral detergent fiber; ADF = acid detergent fiber; Cell = cellulose; Hemi = hemicellulose; Lignin = sulfuric acid lignin; ADIA = acid detergent insoluble Ash; IVTDMD = in vitro true dry matter disappearance; CP = crude protein; NO_3-N = nitrate nitrogen; MSAC = monosaccharides; DSAC = disaccharides; SCPS = short chain polysaccharides; TNC = total nonstructural carbohydrates; MSD = minimum significant difference.

‡ Hays 1, 3, and 5 are PM cuts while Hays 2, 4, and 6 are AM cuts.

differed in much the same way as was found in Exp. 2 (goats). Hemicellulose was lower in the PM cuts but it was the only fiber fraction significantly affected by PM vs. AM cutting. The IVTDMD was again higher in the PM hays, the NO_3-N was lower, and the crude protein did not differ. All the carbohydrate fractions were higher in the PM hays when compared with the paired AM cuts.

Considering only significant effects across experiments, the mean effect of the PM harvest compared with the AM harvest was a 4 g kg^{-1} decrease in hemicellulose, an 18 g kg^{-1} increase in IVTDMD, a 3.7 g kg^{-1} increase in monosaccharides, a 5.0 g kg^{-1} increase in disaccharides, a 0.6 g kg^{-1} increase in short chain polysaccharides, a 1.7 g kg^{-1} increase in starch, and a 10.9 g kg^{-1} increase in TNC. These are relatively minor changes in composition.

Multidimensional Scaling and DMI

A preliminary analysis of the difference values used for multidimensional scaling indicated that the three animal species did not have similar levels of preference among this group of forages. Mean difference values for sheep in Exp. 1 (95% Confidence Limits = 0.15–0.22) and goats in Exp. 2 (95% Confidence Limits = 0.21–0.29) were lower than the difference values for cattle in Exp. 3 (95% Confidence Limits = 0.44–0.57). This indicates that cattle expressed stronger preferences over all comparisons than sheep and goats. These stronger preferences in cattle may have been in part related to the difficulty that cattle have in selecting the preferred portions of the alfalfa hays. Sheep and goats may be more adept at selecting alfalfa leaves from stems. For example, a comparison of the composition of the orts (data not shown) with the as-fed hays showed that goats and sheep had a similar ($P > 0.05$) impact on composition. The fiber fractions were increased while IVTDMD and CP were decreased in the orts. The goats and sheep had overlapping 95% confidence intervals on the change in composition in the orts as compared with the as-fed hay. The net effect on the orts relative to the as-fed hays by goats and sheep was to increase NDF by 97 g kg^{-1}, ADF by 86 g kg^{-1}, cellulose by 69 g kg^{-1}, hemicellulose by 11 g kg^{-1}, and lignin by 17 g kg^{-1}. The IVTDMD was decreased 72 g kg^{-1} and the CP was decreased 58 g kg^{-1}. In contrast, cattle had a smaller net effect (as determined on the basis of 95% confidence intervals, data not shown) on the composition of the orts relative to the as-fed hays. The mean effect on the orts of feeding the hays to cattle was to increase NDF by only 25 g kg^{-1}, ADF by 22 g kg^{-1}, cellulose by 17 g kg^{-1}, hemicellulose by 3 g kg^{-1}, and lignin by 4 g kg^{-1}. The IVTDMD was only decreased 16 g kg^{-1} and the CP was decreased 15 g kg^{-1}. These interrelated factors of varied preference and ability to select preferred por-

Table 5. Composition of alfalfa hays fed to cattle in Exp. 3.

Forage	NDF†	ADF	Cell	Hemi	Lignin	ADIA	IVTDMD	CP	NO_3-N	MSAC	DSAC	SCPS	Starch	TNC
	g kg^{-1} DM													
Hay 1	408	310	239	97	66	3.6	765	221	0.61	12.1	15.5	8.9	6.7	43.2
Hay 2	429	326	251	103	70	4.5	748	216	0.58	10.1	12.7	8.2	5.1	36.2
Hay 3	415	321	247	94	68	4.9	763	221	0.85	13.7	20.7	9.1	6.5	50.0
Hay 4	430	327	251	102	71	4.2	747	224	1.03	9.3	12.8	8.7	4.4	35.3
Hay 5	364	279	214	86	58	5.2	812	231	0.72	17.0	31.4	11.2	6.9	66.5
Hay 6	374	287	220	87	60	5.9	795	229	0.83	12.9	26.6	10.6	4.2	54.3
MSD	31	27	20	6	6	0.7	22	ns	0.09	1.3	2.1	0.4	1.7	2.7
Mean PM	396	303	233	92	64	4.6	780	224	0.73	14.3	22.5	9.7	6.7	53.2
Mean AM	411	313	241	97	67	4.9	763	223	0.81	10.8	17.4	9.2	4.6	41.9
PM vs. AM ($P > F$)	0.09	0.18	0.20	0.01	0.08	0.14	0.02	0.75	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

† Means of six subsamples; NDF = neutral detergent fiber; ADF = acid detergent fiber; Cell = cellulose; Hemi = hemicellulose; Lignin = sulfuric acid lignin; ADIA = acid detergent insoluble Ash; IVTDMD = in vitro true dry matter disappearance; CP = crude protein; NO_3-N = nitrate nitrogen; MSAC = monosaccharides; DSAC = disaccharides; SCPS = short chain polysaccharides; TNC = total nonstructural carbohydrates; MSD = minimum significant difference.

‡ Hays 1, 3, and 5 are PM cuts while Hays 2, 4, and 6 are AM cuts.

tions of the forage on offer must be considered in the interpretation of the MDS and DMI results that follow.

On the basis of a stepwise MDS analysis of residual errors, lack of fit, and the number of estimated parameters associated with 1, 2, and 3 dimensions, all three animal species appeared to base their selection of alfalfa hays on 2 dimensions or criteria with correlation coefficients ranging from 0.63 to 0.76 (Fig. 1). The correlations are calculated between the Euclidian distances between points representing the hays in 2 dimensions (Fig. 1) and the differences (ranging from 0–1) calculated from each animal's relative intakes of each pair of forages. The positions of the points representing the hays in 2 dimensions are based on an iterative least squares fit to the observed differences for each pair of forages, for each animal in the trial. The signs of the coefficients in the dimensions were adjusted to place the more preferred hays in the top right quadrant and the less preferred hays in the bottom left quadrant. However, the first dimension is of greater weight than the second and consequently the position on the *x*-axis should be interpreted as being relatively more important in determining preference than the position on the *y*-axis. The following discussion will also relate the position on the graph to the observed mean DMI (Table 2) but remember that the DMI is a mean intake over all possible pairs. Therefore, DMI expresses preference among all pairs as a single dimension without information relating relative preference for a hay within the context of a specific pair. The 2-dimensional relationships among various pairs are retained in the MDS solution.

The relatively low level of preference among the hays expressed by the sheep (Exp. 1) resulted in a 2-dimensional fit that placed Hay 1 (PM cut) to the left of Hay 2 (AM cut) (Fig. 1). The mean DMI (Table 2) indicated no difference between these two hays in Exp. 1 accounts for the left-right inversion in the fit to the observed difference values and the relatively low correlation between the observed difference values and the distances estimated in the MDS fit. However the graph does reflect the preference for Hay 2 (AM cut) over Hay 4 (AM cut) on the *x*-axis. In addition, Hay 3 (PM cut) and Hay 5 (PM cut) were preferred and this is indicated by their presence in the top right quadrant of the graph. In Exp. 1, we did not find the first dimension to be significantly correlated with any of the estimates of forage composition. In addition, stepwise regression failed to find any model explaining a significant portion of the variation in that dimension (Table 6). On the other hand, MDS dimension 2 was correlated with NDF ($r = -0.84$, $P = 0.03$), hemicellulose ($r = -0.93$, $P = 0.01$), lignin ($r = -0.80$, $P = 0.05$), IVTDMD ($r = 0.88$, $P = 0.02$), disaccharides ($r = 0.93$, $P = 0.01$), short chain polysaccharides ($r = 0.90$, $P = 0.01$), and TNC ($r = 0.92$, $P = 0.01$). Stepwise regression selection for dimension 2 resulted in a model that included nitrate-N and disaccharides and both variables had positive coefficients ($R^2 = 0.97$) (Table 6). For sheep, the nitrogen components of the forage may be important in determining preference after considering the soluble carbohydrates since the stepwise regression for DMI resulted in the selection of crude protein (negative coefficient) and the monosaccharide concentrations (positive coefficient) ($R^2 = 0.97$). Keep in mind that in multiple regression the coefficients only have meaning within the context of the

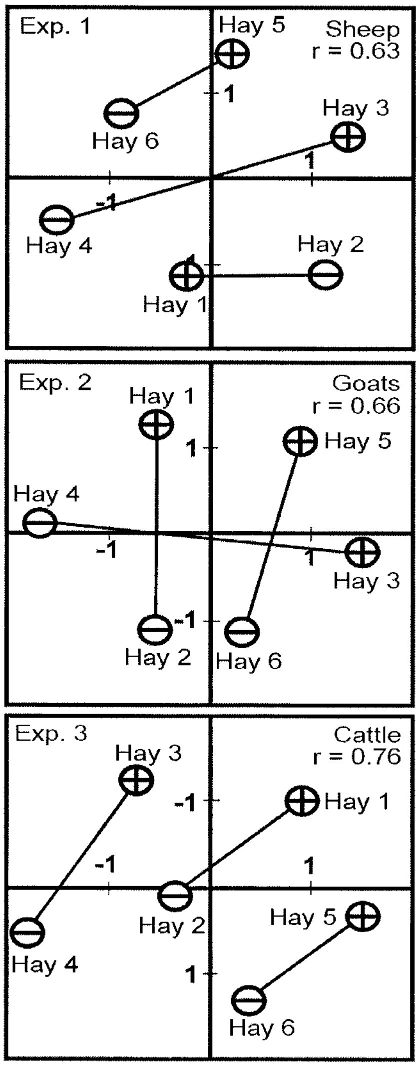

Fig. 1. Results of multidimensional scaling of preference observations from three experiments with more preferred forages located closer to the upper right corner and less preferred forages located closer to the lower left corner. Each pair of hays cut on subsequent days is linked with a line. Circles with plus signs indicate PM hays while circles with minus signs indicate AM hays.

Table 6. Regression Analysis for the prediction of multidimensional scaling dimensions one (Dim 1) and two (Dim 2) and dry matter intake (DMI) in Exp. 1 (sheep).

Dependent variable†	Independent variable	Coefficient	Significance ($P > F$)
Dim 1 =	**None**	**NA**	**NA**
Dim 2 =	**Intercept**	**−3.836**	**<0.01**
	Nitrate-N	**1.810**	**0.04**
	Disaccharides	**0.117**	**<0.01**
			$R^2 = 0.97$
DMI =	**Intercept**	**193.975**	**<0.01**
	Crude Protein	**−4.940**	**0.01**
	Monosaccharides	**21.233**	**<0.01**
			$R^2 = 0.97$

† $n = 6$.

Table 7. Regression Analysis for the prediction of multidimensional scaling dimensions one (Dim 1) and two (Dim 2) and dry matter intake (DMI) in Exp. 2 (goats).

Dependent variable†	Independent variable	Coefficient	Significance ($P > F$)
Dim 1 =	Intercept	−4.333	0.12
	Monosaccharides	0.325	0.11
			$r^2 = 0.51$
Dim 2 =	None	NA	NA
DMI =	Intercept	−17.003	0.73
	Starch	65.248	<0.01
			$r^2 = 0.95$

† $n = 6$.

data set and the other variables in the model. For example, the negative coefficient on crude protein only means that for these data with relatively high crude protein after taking into account carbohydrate level a negative coefficient is associated with protein. It is even possible that protein was selected for the stepwise regression as a surrogate by being correlated with a variable that we didn't measure.

In Exp. 2, MDS analysis indicated that the goats selected the PM over the AM cut in Hays 1 vs. 2 and Hays 5 vs. 6 based almost solely on the second MDS dimension and the PM over the AM in Hays 3 vs. 4 based almost solely on the first dimension (Fig. 1). We found no variables correlated with the first dimension. However, by the more liberal selection criteria of the stepwise procedure, a regression model was selected that included only the concentration of monosaccharides ($r^2 = 0.51$) (Table 7). For dimension 2, no correlations were significant and the stepwise regression did not result in the selection of an equation. However, DMI was correlated with the concentration of monosaccharides ($r = 0.87$, $P = 0.02$) and starch ($r = 0.98$, $P < 0.01$) and stepwise regression resulted in an equation containing only starch ($r^2 = 0.95$). This variation in our ability to explain DMI in contrast to the MDS dimensions is probably due in part to the relatively low levels of preference expressed among the hays in this experiment resulting in relatively poor MDS fits.

Experiment 3 (cattle) gave the best fit of MDS results to observed differences in preference among hays. The cattle had the strongest preferences among the hays and this facilitated the MDS fit. At each cutting the relationship between the AM and PM were similar with the PM harvest located further to the right and higher than the AM harvest; i.e., positive in both dimensions (Fig. 1). The relatively low preference for Hays 3 and 4 is reflected in the low values on the *x*-axis. Dimension 1 was negatively correlated with lignin ($r = -0.82$, $P = 0.05$) and stepwise regression resulted in a model containing lignin and nitrate ($R^2 = 0.95$) (Table 8). Dimension 2 was not correlated with any individual variable but stepwise regression associated the dimension with cellulose and starch ($R^2 = 0.91$) (Table 8). Cellulose had a relatively small positive coefficient and starch had a relatively large positive coefficient. As was found for Dimension 1, DMI was negatively correlated with lignin ($r = -0.87$, $P = 0.02$) and stepwise regression resulted in a model containing lignin and nitrate-N ($R^2 > 0.99$).

Table 8. Regression Analysis for the prediction of multidimensional scaling dimensions one (Dim 1) and two (Dim 2) and dry matter intake (DMI) in Exp. 3 (cattle).

Dependent variable†	Independent variable	Coefficient	Significance ($P > F$)
Dim 1 =	Intercept	13.698	<0.01
	Lignin	−0.164	0.01
	Nitrate-N	−3.866	0.02
			$R^2 = 0.95$
Dim 2 =	Intercept	12.646	0.02
	Cellulose	0.037	0.04
	Starch	0.685	0.02
			$R^2 = 0.91$
DMI =	Intercept	5145.777	<0.01
	Lignin	−52.786	<0.01
	Nitrate-N	−1059.647	<0.01
			$R^2 > 0.99$

† $n = 6$.

SUMMARY

Sheep, goats, and cattle detected subtle differences between hays cut less than 12-h apart and preferred hays cut at sundown over hays cut at dawn. Analysis by multidimensional scaling indicated variation in the criteria used by these animal species to select forages. The sheep and goat experiments each produced a dimension for which we found no correlated composition variables. This may have been due to the poorer MDS fits for the small ruminants. Carbohydrate fractions were present in all the equations selected by stepwise regression for goats and sheep. Nitrate-N and crude protein were included in equations for the goats. In Exp. 3, the first dimension and the DMI of cattle were more closely related to lignin and nitrate-N than any carbohydrate fraction while the carbohydrate fractions were associated with Dimension 2.

Animals were able to identify and select the preferred hays when hays were offered in pairs on days subsequent to a period in which each test hay was offered alone as a meal. Hays cut in the afternoon are of higher nutritive value and were preferred by sheep, goats, and cattle although the selection criteria varied among animal species. Harvesting late in the day is a simple management strategy that can improve forage nutritive value and ruminant preference. When faced with a sunny day and a hay crop ready to cut, producers must balance the need for the extra drying time from mid-morning until late afternoon against the need for a higher quality product.

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Rate of Leaf Appearance in Crimson Clover

Twain J. Butler,* Gerald W. Evers, Mark A. Hussey, and Larry J. Ringer

ABSTRACT

Understanding factors that affect growth and development of crimson clover (*Trifolium incarnatum* L.) are important for the development of management practices to optimize forage utilization. In a 3-yr field experiment at College Station, TX, we evaluated the effects of planting date on rate of leaf appearance of an intermediate- and late-maturing crimson clover. We wanted to determine if growing degree days (GDD) or a photothermal index (PTI) could be used to predict growth. Leaf appearance rates (LAR) did not differ between 'Tibbee' and 'Columbus' crimson clover. Leaf appearance rate was primarily controlled by temperature or GDD, which accounted for 90 to 99% of the variability within each planting date. Photoperiod did not consistently influence the rate of leaf appearance under normal daylengths of 10 h 12 min to 14 h 6 min used in this study. Predictions of LAR were not improved when photoperiod was combined with temperature in a photothermal index than with predictions that used GDD alone. Leaf appearance rate of crimson clover was generally higher when planted in October, November, and December and lower when planted in September, February, and March.

CRIMSON CLOVER is one of the most important clovers for overseeding into perennial warm-season grass sods because of its excellent seedling vigor, early forage production, and early maturity. There have been few studies reported on the growth and development of annual clovers. Leaf appearance rates are direct measurements of morphological development that could be used to quantify factors affecting growth. Understanding and predicting crop growth is beneficial to optimize management, production, and utilization.

Leaf appearance rate on the whole-plant level is a valuable tool for studying growth and development of some plants. Past studies with grasses have utilized phyllochron measurements to quantify morphological development (Kiniry et al., 1991; Van Esbroeck et al., 1997). The phyllochron is defined as the interval in growing degree days (GDD) between the appearance of successive leaves on a stem. Typically, phyllochron research has been done on crops with a single main stem, however, it is often difficult to distinguish a main stem in plants that have a rosette growth habit. Therefore it is more accurate to make comparisons on the whole plant level for such plants.

The GDD is calculated by summing the average daily temperature minus a base temperature. Various species have specific ranges or thresholds of temperature for growth. The base temperature used is the minimum temperature at which a plant species can grow (Eastin and Sullivan, 1984). In addition, a maximum temperature threshold model using an adjusted maximum temperature can account for reduced growth associated with high temperatures (Dufault, 1997). If the maximum daily temperature exceeds an upper limit, the difference between the upper limit and maximum temperature is subtracted from the upper limit to account for the growth reduction.

Strong linear relationships between leaf number and GDD support the contention that temperature is the primary factor controlling leaf appearance rate (LAR) in grasses, soybeans [*Glycine max* (L.) Merrill], peas (*Pisum sativum* L.), cowpeas [*Vigna unguiculata* (L.) Walp.] and subterranean clover (*T. subterraneum* L.) (Baker et al., 1986; Van Esbroeck et al., 1997; Sinclair, 1984; Truong and Duthion, 1993; Ney and Turc, 1993; Crauford et al., 1997; Aitken, 1974). Rate of leaf appearance increases with higher temperatures, but the relationship differs among species.

Photoperiod can have varied effects on LAR. Rate of leaf appearance decreased with increasing photoperiods in glasshouse studies during the spring in certain long-day, cool-season perennial grasses, such as: orchardgrass (*Dactylis glomerta* L.), meadow fescue (*Festuca pratensis* Huds.), perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.), smooth brome (*Bromus inermis* Leyss), and tall fescue (*Festuca arun-*

T.J. Butler, Texas A&M University Agricultural Research and Extension Center, 1229 N. Hwy 281, Stephenville, TX 76401; G.W. Evers, Texas A&M University Agricultural Research and Extension Center, P.O. Box 200, Overton, TX 75684-0200; M.A. Hussey, Soil and Crop Science Dep., and L.J. Ringer, Dep. of Statistics, Texas A&M University, College Station, TX 77843. Received 14 Mar. 2001. *Corresponding author (t-butler@tamu.edu).

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Abbreviations: DL, daylength; LAR, leaf appearance rate; GDD, growing degree days; LN, leaf number; PLS, pure live seed; PTI, photothermal index.