Effects of Fescue Type and Sampling Date on the Nitrogen Disappearance Kinetics of Autumn-Stockpiled Tall Fescue^{1,2}

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ABSTRACT

Two tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] forages, one an experimental host plant/endophyte association containing a novel endophyte that produces low or nil concentrations of ergot alkaloids (HM4) and the other a typical association of Kentucky 31 tall fescue and the wild-type endophyte (*Neotyphodium coenophialum*; E+), were autumn-stockpiled following late-summer clipping and fertilization with 56 kg/ha of N to assess N partitioning and ruminal disappearance kinetics of N for these autumn-stockpiled tall fescue forages. Beginning on December 4, 2003, sixteen 361 ± 56.4-kg replacement dairy heifers were stratified by weight and breeding, and assigned to one of four 1.6-ha pastures (2 each of E+ and HM4) that were strip-grazed throughout the winter. Pastures were sampled before grazing was initiated (December 4), each time heifers were allowed access to a fresh pasture strip (December 26, January 15, and February 4), and when the study was terminated (February 26). Generally, fescue type and the fescue type × sampling date interaction exhibited only minor effects on total forage N, or partitioning of N within the cell solubles or the cell wall. For pregrazed forages, concentrations of N and N partitioned within the cell solubles both declined in a strongly linear relationship with sampling dates. In contrast, concentrations of cell-wall-associated N changed in erratic and often higher-ordered relationships with time, but the magnitude of these responses generally was limited. Unlike the partitioning of N within cell-wall and cell-soluble fractions, kinetic char-

acteristics of ruminal N disappearance frequently exhibited interactions of fescue type and sampling date. For pregrazed forages, these included interactions for all response variables, and for postgrazed forages, fractions B and C, as well as rumen degradable protein. Ruminal disappearance rate for pregrazed E+ and HM4 exhibited quadratic (range = 0.057 to 0.082/h) and cubic (range = 0.057 to 0.075/h) relationships with time, respectively. For postgrazed E+ and HM4 forages, ruminal disappearance rate was unaffected (mean = 0.066/h) or only tended to be affected by sampling date (mean = 0.065/h), respectively. Concentrations of rumen degradable protein exhibited various curvilinear relationships with sampling dates, but disappearance was consistently extensive, and the overall range was relatively narrow (71.3 to 78.9% of N). These findings suggest that ruminal disappearance of N for autumn-stockpiled tall fescue forages remains extensive throughout the winter months and is only affected minimally by fescue type, sampling date, and grazing status.

Key words: grazing, nitrogen disappearance kinetics, replacement heifers, tall fescue

INTRODUCTION

The association of *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon, and Hanlin comb. nov. (Glenn et al., 1996) with its tall fescue host is known to affect the performance of livestock negatively, thereby creating a condition known as fescue toxicosis. Ergot alkaloids produced by this fungus are responsible for various symptoms that may include reduced intakes of DM (Forcherio et al., 1995; Humphry et al., 2002), poorer fiber digestion (Hannah et al., 1990; Humphry et al., 2002), elevated rectal temperatures (Goetsch et al., 1987; McMurphy et al., 1990; Parish et al., 2003), depressed concentrations of serum prolactin (Parish et al., 2003; Nihsen et al., 2004; Watson et al., 2004), rough hair coat (Fribourg et al., 1991; Peters et al., 1992; Nihsen et al., 2004), increased respiration rates (Peters

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et al., 1992; Nihsen et al., 2004), depressed weight gains (Nihsen et al., 2004; Watson et al., 2004; Coblenz et al., 2006b), and reduced milk production in beef cows (Holloway and Butts, 1984; Peters et al., 1992; Coblenz et al., 2006b).

Unfortunately, the same (wild-type) endophyte that affects cattle performance adversely also enhances host-plant competitiveness and persistence, relative to uninfected (endophyte-free) plants (Bouton et al., 1993; West et al., 1993; Malinowski and Belesky, 2000). This is especially relevant throughout the Ozark Highlands, where growing conditions for perennial cool-season grasses are quite stressful. Many Ozark pasture soils are shallow, have poor water-holding capacity, and are often acidic with relatively low fertility (Sauer et al., 1998). In addition, most pastures throughout this region also contain significant percentages of bermudagrass [*Cynodon dactylon* (L.) Pers.] that can compete aggressively with tall fescue throughout the summer months (Coblenz et al., 2006a).

Recently, forage scientists have identified novel endophytes that produce minimal or no measurable concentrations of ergot alkaloids when they are associated with host fescue plants (Bouton et al., 2002; Nihsen et al., 2004), and these associations appear to alleviate most of the classical symptoms of fescue toxicosis in livestock (Parish et al., 2003; Nihsen et al., 2004; Watson et al., 2004). These observations are encouraging and are coupled with cautious optimism that the symbiotic relationships that support superior plant persistence and stand survival also will be retained.

Whereas much of the existing tall fescue or livestock research, or both, has evaluated plant and animal performance during the spring and summer months, there has been increased interest in autumn stockpiling tall fescue forage for grazing livestock during winter (Poore et al., 2000; Kallenbach et al., 2003; Teutsch et al., 2005). The creation of new feeding models for livestock (Sniffen et al., 1992; NRC, 1996, 2001) has resulted in a need for in-depth knowledge of forage proteins. Understanding the distribution of forage N within fiber and cell-soluble fractions, as well as the ruminal disappearance kinetics of forage N, are important considerations for obtaining the greatest benefit from these feeding models. Currently, there is little research information available that describes the partitioning of N within cell-soluble (NDSN) and cell-wall (NDIN) fractions, or the kinetics of ruminal N disappearance for autumn-stockpiled tall fescue forages. Moreover, relatively little is known about how kinetic parameters may be affected by specific associations of host plant and endophyte, grazing by livestock, or both. Our objectives were to evaluate N partitioning and in situ disappearance kinetics of N for pregrazed and postgrazed au-

tumn-stockpiled tall fescue forages sampled on 5 dates throughout the winter in the southern Ozark Highlands.

MATERIALS AND METHODS

Complete descriptions of pastures, agronomic management, weather data, forage mass, concentrations of ergovaline, animal care and management, as well as fiber composition and in situ disappearance kinetics of NDF and DM have been reported previously (Flores et al., 2007).

Pastures and Management

Briefly, four 1.6-ha pastures located at the Arkansas Agricultural Research and Extension Center in Fayetteville were established in 1998 with 1) an association of tall fescue with a novel endophyte that produces low or nil concentrations of ergot alkaloids (**HM4**; Nihsen et al., 2004), or 2) an association of Kentucky 31 tall fescue with the wild-type endophyte (*Neotyphodium coenophialum*; **E+**), which is observed commonly throughout the Ozark Highlands. Each tall fescue type was established within 2 of the 4 experimental pastures. Pastures were clipped to a common 7.5-cm stubble height with a rotary mower on September 9, 2003, and then fertilized at a rate of 56 kg of N/ha with ammonium nitrate (34-0-0) the following day. After fertilizing with N, no animals were allowed to graze these experimental pastures until the initiation of the research trial on December 4, 2003.

Grazing Management

On December 4, 2003, sixteen 361 ± 56.4 -kg dairy heifers were stratified by weight and breed type (Holstein or Jersey $\times$ Holstein), and assigned to 1 of the 4 experimental pastures (4 heifers per pasture). These replacement heifers were utilized solely to apply grazing pressure, and to create grazed forages that could be sampled for subsequent evaluations of nutritive value and ruminal disappearance kinetics of N. During this time, heifers had ad libitum access to fresh water and were offered a corn-based concentrate supplement on a group basis at 1700 h each day at a rate equal to 2.0 kg/d for each individual heifer.

Experimental pastures were strip-grazed using techniques observed commonly throughout the region. On December 4, heifers in each pasture were allowed to strip graze a 0.4-ha area, or approximately 25% of each pasture. Heifers were allowed to graze this initial strip for about 21 d, and a single lead electric wire was used to limit access to the remaining 75% of the 1.6-ha pas-

ture. On December 26, January 15, and February 4, heifers in each pasture were allowed access to an additional 0.4 ha (25%) of each 1.6-ha pasture by advancing the lead electric wire. No back wire was used; therefore, heifers had continued access to all stale (postgrazed) strips after the lead electric wire was advanced. Grazing was terminated on February 26, after heifers had spent a total of 84 d on pasture.

Pasture Sampling

Pregrazed Forages. Pastures were sampled on days that grazing heifers were allowed access to a new strip (December 4, December 26, January 15, and February 4), and when grazing was terminated (February 26). On December 4, forage was obtained by clipping all the forage within four 0.25-m² frames to a 2.5-cm stubble height with garden shears. Frames were placed randomly throughout the 0.4-ha strip that heifers entered when the trial was initiated. For the December 26, January 15, and February 4 sampling dates, pregrazed forages were sampled in an identical manner from the fresh strips that heifers were entering for the first time on those dates. On the date that grazing was terminated (February 26), pregrazed forage was obtained by clipping 0.25-m² frames from within 4 circular (1.6-m diameter) enclosures that were positioned at random before grazing was initiated.

Postgrazed Forages. Postgrazed forages were sampled similarly from 4 random locations within the strip that heifers were exiting on December 26, January 15, February 4, and when grazing was terminated (February 26). To ensure that there was adequate postgrazed forage for the subsequent planned analyses, a paired set of 0.25-m² frames (located within 2 m of each other) were clipped at each of 4 random locations within each grazed strip.

Laboratory Analysis of Forages

Clipped forages were dried to constant weight under forced air at 50°C, and then ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) fitted with a 1- or 2-mm screen. Portions of each sample ground through a 2-mm screen were stored in sealed plastic bags, and retained for subsequent ruminal incubation in situ. Forage samples that were ground through a 1-mm screen were analyzed for total N, NDIN, and ADIN. To determine NDIN and ADIN, forages were digested in neutral or acid detergent using batch procedures outlined by Ankom Technology Corp. (Fairport, NY) for an Ankom 200 Fiber Analyzer. Neither sodium sulfite (Van Soest et al., 1991; Licitra et al., 1996) nor heat-stable α -amylase was included in the NDF solution.

For ADIN, digestion of forages in acid detergent was conducted nonsequentially, without a preliminary digestion in neutral detergent (Van Soest et al., 1991). Concentrations of total N in the original forages, as well as in residues following digestion in neutral and acid detergent, were determined by rapid combustion (AOAC, 1998, official method 990.03; Elementar Americas Inc. Mt. Laurel, NJ). Concentrations of NDIN and ADIN were reported as percentages of total forage DM and N. The concentration of NDSN was calculated as the difference between total N and NDIN (% of DM).

In Situ Incubation of Experimental Forages

Animal Care. Five 565 $\pm$ 35.1-kg ruminally cannulated crossbred (Gelbvieh $\times$ Angus $\times$ Brangus) steers were used to conduct ruminal incubations in situ. Steers were housed in individual 3.4 $\times$ 4.9-m pens with concrete floors that were cleaned regularly and were offered a basal diet consisting of alfalfa hay (20.7% CP, 49.2% NDF, and 37.7% ADF) and cracked corn. On an as-fed basis, the basal diet contained 85.0% alfalfa hay and 14.8% cracked corn. Trace mineralized salt, which was top-dressed over the cracked corn at each feeding, composed the balance (0.2%) of the total basal diet. The diet was offered in equal portions at 0700 and 1700 h for a daily DMI of 2.25% of BW. All steers consumed this daily allotment without refusal. Fresh water was available continuously, and steers were adapted to the basal diet for 10 d before initiating the trial. Cannulations and care of the steers were approved by the University of Arkansas Animal Care and Use Committee (protocol #05005).

Kinetic Procedures. All ruminal incubation procedures have been described previously (Flores et al., 2007). A total of 18 treatment combinations were evaluated simultaneously in the cannulated steers. These included 10 pregrazed forages clipped from 2 fescue types (HM4 and E+) on 5 sampling dates, and 8 postgrazed forages that were clipped from both fescue types on the final 4 sampling dates. To restrict in situ incubations to a manageable number of forages, each treatment combination was composited over like field replications (pastures) before conducting ruminal incubations.

In situ procedures were consistent with the standardized techniques described by Vanzant et al. (1998). Five-gram samples were weighed into Dacron bags (10 cm $\times$ 20 cm; 50 $\pm$ 10- μ m pore size; Ankom Technology Corp.) that were heat sealed with an impulse sealer (Type TISH-200; TEWI International Co. Ltd., Taipei, Taiwan). Bags were then placed in 35 $\times$ 50-cm mesh bags, incubated in tepid water (39°C) for 20 min, and then suspended in the ventral rumen immediately prior to

the 0700-h feeding for 3, 6, 9, 12, 24, 36, 48, 72, or 96 h. Upon removal from the rumen, bags were washed immediately with 10 cold-water rinse cycles (Coblentz et al., 1997; Vanzant et al., 1998) in a top-loading washing machine (model LXR7144EQ1; Whirlpool Corp., Benton Harbor, MI). An additional set of bags was rinsed without ruminal incubation (0 h). After rinsing, all residues were dried to a constant weight at 50°C and equilibrated with the atmosphere before determination of residual DM (Vanzant et al., 1996). Concentrations of N within the residual forage contents of each bag were determined by the combustion procedure described previously.

The percentage of N remaining at each incubation time was fitted to the nonlinear regression model of Mertens and Loften (1980) using PROC NLIN of SAS (1990). Forage N was partitioned into 3 fractions based on relative susceptibility to ruminal disappearance. Fraction A was defined as the immediately soluble portion of the total N pool, which disappears from bags at rates inestimable by these procedures. Within the rumen, this N is assumed to be converted rapidly into ammonia, although some exceptions to this generalization are known to occur (Broderick, 1994). Fraction B represents the percentage of N that disappears at a measurable rate, whereas fraction C is the percentage of the total N pool that is unavailable in the rumen. Fractions B and C, disappearance rate (**Kd**), and discrete lag time were determined directly by the nonlinear regression model. For each forage, fraction A was calculated as $100\% - (B + C)$, and rumen degradable protein (**RDP**) was calculated as $A + B \times [Kd / (Kd + Kp)]$ (Ørskov and McDonald, 1979), where Kp = passage rate. Ruminal passage rate (mean = $0.026 \pm 0.0036 \text{ h}^{-1}$) of the basal diet was determined for each steer using acid-detergent-insoluble ash as an internal passage marker (Flores et al., 2007). Calculations of RDP for each individual steer were based on the Kp determined experimentally in that same steer during the trial.

Before calculating disappearance kinetics, a subset ($n = 42$) of in situ residues selected with representation from all steers, forages, and incubation periods were analyzed for concentrations of purines by the method of Zinn and Owens (1986) to assess levels of microbial contamination. Purine concentrations were found to be negligible (overall mean = $0.08 \pm 0.042\%$ of DM), and no corrections for microbial contaminant N were made before calculating kinetics of ruminal N disappearance.

Statistics

N Components. Because the number of sampling dates differed with grazing status, pre- and postgrazed forages were analyzed within independent split-plot de-

signs. In both cases, tall fescue type (E+ or HM4) was the whole-plot term, whereas sampling date served as the subplot treatment effect. Whole-plot effects were tested for significance with the pasture nested within fescue type error mean square by PROC GLM of SAS (SAS Institute, 1990). Sampling dates and the interaction of fescue type $\times$ sampling date were tested for significance with the residual error mean square. Single-degree-of-freedom orthogonal contrasts were used to evaluate N components for linear, quadratic, cubic, or quartic effects of time.

In Situ Disappearance of N. Kinetic characteristics were analyzed as a randomized complete block design with the 5 steers serving as experimental blocks. For pregrazed forages, treatment factors were evaluated as a 2×5 factorial arrangement of fescue types and sampling dates. For postgrazed forages, a similar ANOVA was conducted that included both fescue types, but only 4 sampling dates. As described for N components, single-degree-of-freedom orthogonal contrasts were used to evaluate kinetic indices for linear, quadratic, cubic, or quartic effects of time.

Comparisons of Pregrazed and Postgrazed Forages. For the final 4 sampling dates, N components and kinetic characteristics for pregrazed forages were compared with those of postgrazed forages by creating a new variable calculated as the difference between estimates (for example, $NDF_{\text{pregrazed}} - NDF_{\text{postgrazed}}$). This difference was subsequently compared with zero using a Student's *t*-test (PROC GLM; SAS Institute, 1990). In all cases, significance was declared at $P \leq 0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

Nitrogen Partitioning

Pregrazed Forages. Excluding ADIN (% of DM; $P = 0.011$), fescue type did not affect any aspect of N partitioning ($P \geq 0.657$; Table 1). Similarly, the interaction of main effects did not affect the measured concentration of any N component ($P \geq 0.356$); however, all N components were affected by sampling date ($P \leq 0.022$). For these reasons, only sampling date means are presented and discussed (Table 2).

Concentrations of total N and NDSN both declined linearly ($P = 0.001$) over sampling dates. By the final sampling date on February 26, total N declined by 0.45 percentage units (18%) from the initial concentration (2.50%) on December 4. Over the same time period, a similar reduction (0.48 percentage units) was observed for NDSN; however, this constituted a larger percentage of the initial NDSN pool (29%). Concentrations of N for these autumn-stockpiled forages corresponded closely to those reported by Teutsch et al. (2005; 2.22%)

Table 1. Abbreviated ANOVA for indices of nutritive value for pregrazed and postgrazed autumn-stockpiled tall fescue forages (HM4 or E+) sampled on 5 dates between December 4, 2003, and February 26, 2004

Source of variation	N, % of DM	NDSN, ¹ % of DM	NDIN, % of DM	NDIN, % of N	ADIN, % of DM	ADIN, % of N
<i>P > F</i>						
Pregrazed forage						
Fescue type (F)	0.980	0.965	0.981	0.786	0.011	0.657
Sampling date (D)	0.006	0.005	0.022	0.002	0.003	0.002
F × D	0.356	0.399	0.851	0.408	0.567	0.479
Postgrazed forage						
Fescue type (F)	0.512	0.440	0.689	0.083	0.701	0.496
Sampling date (D)	0.166	0.072	0.010	0.007	0.006	0.010
F × D	0.145	0.211	0.224	0.312	0.749	0.408

¹Abbreviations: NDSN, neutral-detergent soluble N; NDIN, neutral-detergent insoluble N; and ADIN, acid-detergent insoluble N.

for stockpiled tall fescue fertilized with 45 kg of N/ha and harvested in mid December. Comparable concentrations of total N also were reported by Kallenbach et al. (2003; 2.13%) for stockpiled fescue harvested in Missouri at approximately the same time period.

Concentrations of NDIN, expressed on a DM or total N basis, changed in a cubic ($P \leq 0.039$) pattern over sampling dates. From a practical standpoint, these responses were limited when expressed on a percentage of DM basis, exhibiting a narrow range over all sampling dates (0.79 to 0.93%); furthermore, concentrations were nearly identical on the initial and final sampling dates (0.82 and 0.85%, respectively). Expressed as a percentage of total N, NDIN increased from 33.5% of N on the initial sampling date to 43.8% of N on February 4, but these responses were largely a function of declining concentrations of total N, rather than an expanding pool of N associated with the cell wall. Previously, Elizalde et

al. (1999) reported concentrations of NDIN ranging from 14.3 to 24.9% of total N for endophyte-free and endophyte-infected tall fescue forages harvested at the tillering, stem elongation, heading, and flowering stages of growth during spring. Relative to our study, these substantial differences for cell-wall-associated N may be related to more aggressive spring fertilization management (100 kg of N/ha in early April), physiological differences between fall/winter and spring growth, or a combination of these factors. Acid-detergent insoluble N also changed in a cubic ($P \leq 0.003$) pattern over sampling dates, exhibiting minimum concentrations (0.132% of DM, 6.11% of N) on December 26 and maxima (0.211% of DM; 10.19% of N) on February 4.

Postgrazed Forages. No response variable was affected by fescue type ($P \geq 0.083$) or the interaction of main effects ($P \geq 0.145$; Table 1); therefore, only sampling date means are presented and discussed (Table

Table 2. Concentrations of N components for pregrazed (PRE) and postgrazed (POST) autumn-stockpiled tall fescue forages sampled on 5 dates between December 4, 2003, and February 26, 2004

Date	N		NDSN ¹		NDIN		NDIN		ADIN		ADIN	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
	% of DM				% of N				% of DM			
Dec 4	2.50	—	1.68	—	0.82	—	33.5	—	0.157	—	6.48	—
Dec 26	2.32	2.22	1.53	1.43	0.79	0.80	34.6	36.0	0.132	0.141	6.11	6.44
Jan 15	2.22	2.05	1.29	1.15	0.93	0.90	41.9	44.3	0.182	0.184	8.25	9.07
Feb 4	2.10	2.22	1.19	1.34	0.91	0.89	43.8 ^a	40.0 ^b	0.211	0.228	10.19	10.29
Feb 26	2.05	2.02	1.20	1.20	0.85	0.83	41.2	40.8	0.170	0.159	8.46	7.89
SEM	0.064	0.069	0.074	0.064	0.025	0.016	1.39	1.01	0.0091	0.0108	0.472	0.523
Contrast ²	<i>P > F</i>											
Linear	0.001	0.229	0.001	0.138	0.079	0.326	0.001	0.063	0.006	0.092	0.001	0.055
Quadratic	0.324	0.867	0.126	0.311	0.046	0.002	0.041	0.010	0.174	0.002	0.136	0.003
Cubic	0.998	0.059	0.383	0.032	0.027	0.326	0.039	0.008	0.001	0.057	0.003	0.379
Quartic	0.745	—	0.686	—	0.071	—	0.318	—	0.552	—	0.845	—

^{a,b}Within each index of nutritive value and sampling date, means without common superscripts differ at $P \leq 0.05$.

¹Abbreviations: NDSN = neutral-detergent soluble N; NDIN = neutral-detergent insoluble N; and ADIN = acid-detergent insoluble N.

²Linear, quadratic, cubic, and quartic effects of sampling date.

Table 3. Abbreviated ANOVA for characteristics of ruminal N disappearance for pregrazed and postgrazed autumn-stockpiled tall fescue forages (HM4 or E+) sampled on 5 dates between December 4, 2003, and February 26, 2004

Source of variation	Fraction ¹			Lag time	Kd	RDP ²
	A	B	C			
Pregrazed forage	<i>P > F</i>					
Fescue type (F)	0.110	0.043	0.117	0.519	0.630	0.901
Sampling date (D)	<0.001	<0.001	<0.001	0.043	0.316	<0.001
F × D	<0.001	<0.001	<0.001	0.005	0.004	<0.001
Postgrazed forage						
Fescue type (F)	0.569	0.302	0.130	0.625	0.779	0.600
Sampling date (D)	0.055	0.001	<0.001	0.715	0.412	0.007
F × D	0.211	0.001	<0.001	0.915	0.105	0.015

¹Abbreviations: A = immediately soluble fraction, B = fraction degradable at a measurable rate, C = undegradable fraction, Kd = fractional degradation rate, RDP = effective ruminal degradability of CP.

²Calculated as $A + (B \times [Kd/Kd + \text{passage rate}])$, where Kd = ruminal degradation rate and passage rate = $0.026 \pm 0.0036/\text{h}$ (Flores et al., 2007).

2). Total N only tended ($P = 0.059$) to decline in a cubic pattern over sampling dates, but the overall range was relatively small (2.22 to 2.02% of DM). Similarly, concentrations of NDSN decreased in a cubic ($P = 0.032$) pattern that largely mirrored responses for total N over sampling dates. Concentrations of NDIN (% of DM; $P = 0.002$) and ADIN ($P \leq 0.003$) changed quadratically over time; however, minimum numerical concentrations of each were observed on December 26, whereas the maximum concentrations were observed on January 15 or February 4. In each case, concentrations observed on the final sampling date differed only minimally from those on the initial date. Expressed as a percentage of total N, NDIN increased cubically ($P = 0.008$) from 36.0 to 44.3% of N by December 26 before declining to 40.0 and 40.8% of N on the final 2 sampling dates.

Pre- and postgrazed forages did not differ ($P > 0.05$) for any response variable on any sampling date, except for NDIN (43.8 vs. 40.0% of N, $P \leq 0.05$) on February 4, thereby indicating only minor effects of grazing on N partitioning within these forages throughout the winter.

In Situ Disappearance Kinetics

Pregrazed Forages. Fescue type had relatively little effect on kinetic characteristics, exhibiting nonsignificant ($P \geq 0.110$) effects for all response variables except fraction B ($P = 0.043$). However, all kinetic characteristics (fractions A, B, and C, lag time, Kd, and RDP) exhibited strong fescue type × sampling date interactions ($P \leq 0.005$; Table 3). Therefore, only interaction means are presented and discussed. This overall limited response to endophyte status is consistent with a previous report (Elizalde et al., 1999) in which no differences were observed between endophyte-infected and endophyte-free tall fescue for fraction B, potential

extent, Kd, and RDP when harvests were made at 4 stages of growth during spring.

For E+ fescue (Table 4), all kinetic characteristics changed in curvilinear ($P \leq 0.015$) relationships with sampling dates, and all but Kd exhibited complex cubic or quartic effects (Table 4). Despite the cubic ($P = 0.015$) relationship with sampling dates, concentrations of RDP changed only marginally, ranging from 74.0% on December 26 and January 15 up to 77.2% on February 4. Furthermore, estimates on the initial and final sampling dates differed by only 0.3 percentage units, thereby indicating that RDP was essentially stable over the entire winter sampling period. Although fraction C exhibited a complex quartic ($P < 0.001$) effect, the magnitude of this response also was only modest, ranging from 10.7 to 14.5% of N over 5 sampling dates, and suggests that this pool of forage N was fairly static throughout the winter. In contrast, relatively large shifts in percentages of total N were observed over sampling dates for fraction A (range = 15.3 percentage units) and B (range = 18.4 percentage units). Although a strong cubic ($P < 0.001$) effect was observed for both of these kinetic characteristics, these shifts were apparently confined to highly rumen degradable forms because they had little practical effect on estimates of RDP. Estimates of lag time exhibited a quartic ($P = 0.012$) response over sampling dates that included a maximum of 3.59 h on January 15. Similarly, Kd reached a maximum (0.082/h) on the same date; however, estimates for December 4, December 26, and February 26 were virtually identical (0.061, 0.061, and 0.057/h, respectively).

For HM4 tall fescue (Table 5), fractions A, B, and C, as well as RDP, all changed in complex quartic ($P \leq 0.002$) patterns over winter. Responses for lag time and Kd also were higher-ordered, both exhibiting cubic ($P \leq 0.021$) patterns. As described for E+ fescue, the practi-

Table 4. Ruminal disappearance kinetics of N for pregrazed (PRE) and postgrazed (POST) autumn-stockpiled E+ tall-fescue forage sampled on 5 dates between December 4, 2003, and February 26, 2004

Date	Fraction ¹						Lag time		Kd		RDP ²	
	A		B		C							
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
	% of N						h		/h		% of N	
Dec 4	42.6	—	46.2	—	11.3	—	0.75	—	0.061	—	74.9	—
Dec 26	37.0	40.0	52.3	48.1	10.7	11.8	0.75	2.12	0.063	0.060	74.0	73.8
Jan 15	38.6	43.0	46.9	44.5	14.5 ^a	12.4 ^b	3.59 ^a	1.75 ^b	0.082	0.071	74.0	75.4
Feb 4	52.3 ^a	45.3 ^b	33.9	37.3	13.8 ^b	17.5 ^a	2.13	1.82	0.075	0.064	77.2 ^a	71.6 ^b
Feb 26	48.1	44.9	39.5	40.9	12.4 ^b	14.2 ^a	1.39	1.91	0.057	0.067	75.2	74.5
SEM	2.06	1.28	2.03	1.33	0.34	0.39	0.513	0.537	0.0055	0.0041	0.68	0.62
Contrast ³	$P > F$											
Linear	0.001	0.013	<0.001	<0.001	<0.001	<0.001	0.120	0.824	0.856	0.432	0.095	0.594
Quadratic	0.076	0.212	0.281	0.018	<0.001	<0.001	0.008	0.677	0.004	0.340	0.690	0.308
Cubic	0.001	0.741	<0.001	0.030	<0.001	<0.001	0.209	0.857	0.148	0.169	0.015	0.001
Quartic	0.061	—	0.206	—	<0.001	—	0.012	—	0.221	—	0.087	—

^{a,b}Within each index of nutritive value and sampling date, means without common superscripts differ at $P \leq 0.05$.

¹Abbreviations: A = immediately soluble fraction, B = fraction degradable at a measurable rate, C = undegradable fraction, Kd = fractional degradation rate, RDP = effective ruminal degradability of CP.

²Calculated as $A + (B \times [Kd/Kd + \text{passage rate}])$, where Kd = ruminal degradation rate and passage rate = $0.026 \pm 0.0036/\text{h}$ (Flores et al., 2007).

³Linear, quadratic, cubic, and quartic effects of sampling date.

cal relevance of most of these statistically significant responses is somewhat questionable. Whereas some shifts in partitioning between fractions A, B, and C occurred over sampling dates, these shifting pools had only modest effects on estimates of RDP. Respective estimates of RDP decreased from 78.9 to 71.3% between December 4 and January 15 before increasing slightly thereafter. Generally, this represents a relatively sta-

ble response over time, and within that context, is consistent with responses for E+ that ranged between 74.0 and 77.2% of N over the same time period.

Although relatively little information is available that describes N partitioning and rumen disappearance kinetics of N from autumn-stockpiled tall fescue forages, our estimates of RDP for these forages are comparable with those made for early spring growth by others

Table 5. Ruminal disappearance kinetics of N for pregrazed (PRE) and postgrazed (POST) autumn-stockpiled HM4 tall-fescue forage sampled on 5 dates between December 4, 2003, and February 26, 2004

Date	Fraction ¹											
							Lag time		Kd		RDP ²	
	A		B		C							
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
	% of N						h		/h		% of N	
Dec 4	49.8	—	41.1	—	9.1	—	0.58	—	0.063	—	78.9	—
Dec 26	48.0 ^a	42.3 ^b	40.2	44.0	11.8 ^b	13.7 ^a	2.23	2.01	0.075	0.072	77.7 ^a	74.5 ^b
Jan 15	40.7	42.7	43.6	41.9	15.7	15.4	1.15	1.55	0.062	0.063	71.3	72.4
Feb 4	49.9 ^a	43.9 ^b	35.9 ^b	42.8 ^a	14.2	13.3	0.93	1.35	0.057	0.057	74.5	72.9
Feb 26	40.8	42.1	43.6	45.8	15.6 ^a	12.1 ^b	2.77	2.19	0.070	0.067	72.6 ^b	75.1 ^a
SEM	1.17	1.08	1.35	1.11	0.40	0.48	0.592	0.535	0.0050	0.0046	0.57	0.72
Contrast ³	$P > F$											
Linear	0.001	0.921	0.860	0.223	<0.001	0.007	0.119	0.892	0.824	0.325	<0.001	0.503
Quadratic	0.657	0.307	0.232	0.037	<0.001	0.011	0.587	0.245	0.532	0.060	0.002	0.012
Cubic	0.003	0.455	0.020	0.866	0.170	0.053	0.021	0.749	0.013	0.523	0.939	0.758
Quartic	<0.001	—	0.002	—	<0.001	—	0.631	—	0.586	—	<0.001	—

^{a,b}Within each index of nutritive value and sampling date, means without common superscripts differ at $P \leq 0.05$.

¹Abbreviations: A = immediately soluble fraction, B = fraction degradable at a measurable rate, C = undegradable fraction, and Kd = fractional degradation rate, RDP = effective ruminal degradability of CP.

²Calculated as $A + (B \times [Kd/Kd + \text{passage rate}])$, where Kd = ruminal degradation rate and passage rate = $0.026 \pm 0.0036/\text{h}$ (Flores et al., 2007).

³Linear, quadratic, cubic, and quartic effects of sampling date.

(Elizalde et al., 1999; Dubbs et al., 2003). Specifically, our overall range of estimates (71.3 to 78.9%) is very similar to in situ estimates for endophyte-free fescue made at the tillering, stem elongation, heading, and flowering stages of growth (69.9 to 82.4%), and for endophyte-infected fescue at the tillering, stem elongation, and heading stages of growth (73.4 to 81.7%; Elizalde et al., 1999). Similarly, Dubbs et al. (2003) used the *Streptomyces griseus* procedure and reported estimates of RDP of 72.4 and 74.6% for masticate samples obtained from vegetative growth in grazed pastures during April and October, respectively. Our estimates of RDP for autumn-stockpiled forages also correspond closely to estimates for orchardgrass (*Dactylis glomerata* L.) made at various stages of spring growth (71.4 to 81.7%, Hoffman et al., 1993; 69.6 to 78.7%, Balde et al., 1993). Although these comparisons suggest that estimates of RDP for autumn-stockpiled tall fescue are generally comparable to those observed typically for early spring growth, they represent considerably greater estimates than those observed for hays composed of fully headed tall fescue. Turner et al. (2004) reported estimates of RDP ranging from 32.6 to 49.1% for tall fescue hays harvested in Arkansas during late May. These hays were damaged by rainfall during wilting, spontaneous heating, or both during storage, or they were harvested and stored without these forms of suboptimal management. These types of production problems, often coupled with harvest delays during periods of wet or threatening weather, are common during spring throughout the southern Ozarks, and hays harvested under these conditions compose a significant portion of the supplemental feed offered to beef cows or other livestock during the following winter months.

Postgrazed Forages. There were no differences ($P \geq 0.130$; Table 3) across fescue types for any response variable; however, sampling date, as well as the fescue type $\times$ sampling date interaction exhibited significant ($P \leq 0.015$) effects for fractions B and C, and RDP. Because fescue type $\times$ sampling date interactions were observed for 3 of 6 response variables, sampling date responses are presented and discussed by fescue type.

For E+ fescue (Table 4), fraction A increased in a linear ($P = 0.013$; Table 4) relationship with time; estimates differed numerically by 4.9 percentage units on December 26 and February 26. Fractions B and C, as well as RDP, changed in a cubic ($P \leq 0.030$) pattern over sampling dates. For fraction B, this generally represented a declining pattern with a 7.2-percentage unit differential between the December 26 and February 26 sampling dates. In contrast, fraction C generally increased (from 11.8 to 17.5%) between December 26 and February 4 before decreasing slightly on the final sampling date. Estimates of lag time (overall mean =

1.90 h) and Kd (overall mean = 0.066/h) were not affected by sampling date ($P \geq 0.169$). As observed for pregrazed E+ fescue, statistically significant shifts in fractions A, B, and C had relatively little practical effect on estimates of RDP. Although RDP exhibited a cubic ($P = 0.001$) relationship with sampling dates, the range over dates was small (71.6 to 75.4%) and estimates on December 26 and February 26 differed by only 0.7 percentage units.

For postgrazed HM4 tall fescue (Table 5), sampling date had no effect on fraction A (overall mean = 42.8%; $P \geq 0.307$), lag time (overall mean = 1.78 h; $P \geq 0.245$), or Kd (overall mean = 0.065/h; $P \geq 0.060$). In contrast, fractions B and C, and RDP all changed in quadratic ($P \leq 0.037$) patterns over the winter; in practical terms, these quadratic responses did not depict forages whose nutritional values were changing rapidly, or that were especially sensitive to weathering. For each of these kinetic characteristics, the overall range across dates was narrow (3.9, 3.3, and 2.7 percentage units for fractions B and C, and RDP, respectively) with especially small differentials between the December 26 and February 26 sampling dates.

Pregrazed vs. Postgrazed Forages. For both E+ (Table 4) and HM4 (Table 5) tall fescue, there were relatively few differences between pregrazed and postgrazed forages, indicating that grazing status had little practical effect on kinetic estimates. For E+, the sharpest differences ($P \leq 0.05$) occurred on February 4 for fraction A (7.0 percentage units) and RDP (5.6 percentage units). The percentage of total forage N that was unavailable in the rumen (fraction C) differed ($P < 0.05$) on the basis of grazing status on 3 dates (January 15, February 4, and February 26); however, this fraction was greatest ($P < 0.05$) for pregrazed forage on January 15, but smaller ($P < 0.05$) before grazing on the final 2 sampling dates. For HM4, the sharpest differences ($P < 0.05$) between pre- and postgrazed forages occurred on February 4. On that sampling date, pregrazed forages partitioned a greater percentage of total N into fraction A (49.9 vs. 43.9%) and a smaller percentage into fraction B (35.9 vs. 42.8%) than did postgrazed forages; however, this shift among N pools had no effect ($P > 0.05$) on respective estimates of RDP (74.5 vs. 72.9%).

Implications

In practical terms, none of the treatment factors (fescue type, sampling date, or grazing status) had a large practical effect on N partitioning or ruminal N disappearance kinetics of autumn-stockpiled tall fescue forages. Although there were statistically significant responses to treatment, these were generally confined to relatively small ranges. Regardless of treatment, con-

centrations of N in stockpiled forages remained >2.0%, which was confined primarily within highly rumen degradable forms. Estimates of effective ruminal degradability ranged from 71.3 to 78.9%, which generally are comparable with other reports for early-spring growth of tall fescue and other perennial cool-season grasses. Autumn-stockpiled tall fescue from the southern Ozark Highlands would likely be offered to cattle over winter in lieu of fully headed hays that were made during late spring and stored until winter. Within this context, autumn-stockpiled fescue forage will likely contain greater concentrations of N that exists in forms that are more available ruminally than most tall fescue hays. A recent survey (Davis et al., 2002) of tall fescue hays produced in Arkansas between 1985 and 1999 (n = 908) indicated that concentrations of CP ranged from 3.9 to 22.4%, but averaged only 11.2% over this extended time period. Even if this CP was distributed ideally between RDP and RUP pools, it would not generally be adequate for dairy heifers growing at a rate of 0.8 kg/d, regardless of breed type, reproductive status, or BW (NRC, 2001).

Despite the highly desirable nutritive and kinetic traits exhibited by these autumn-stockpiled tall fescue forages, some caution is advised with respect to interpretation. A review of previous work (Poore et al., 2000) suggests that the performance of growing livestock in grazing trials has frequently fallen below expectations based on the relatively high quality characteristics exhibited by these stockpiled forages. This disappointing performance may be related to poor voluntary intake by grazing animals. One study (Poore et al., 2006) estimated the intake of forage OM by growing beef heifers over 2 yr at about 1.2 and 1.6% of BW. Based on removal rates of forage DM reported previously for the present study (Flores et al., 2007), voluntary intakes of forage DM ($\approx$ 1.2% of BW daily) appeared to be consistent with past estimates.

Forages that self-limit consumption might be highly desirable for maintaining pregnant, nonlactating beef cows that have low nutritional requirements, but would be unsuitable for developing dairy heifers. Furthermore, a nonpregnant 300-kg replacement (large breed) dairy heifer gaining 0.8 kg/d requires 685 g of RDP/d (NRC, 2001). Assuming the mean concentrations of CP (14.1%) and RDP (74.5%) obtained from our study, a 300-kg replacement heifer would need to consume stockpiled fescue at a rate of about 2.2% of BW daily to meet this requirement without additional supplementation. Based on the cautions described by Poore et al. (2000, 2006) and the forage removal from our pastures summarized previously (Flores et al., 2007), this rate of intake seems quite unlikely. It also remains unclear how endophyte status (wild type, novel, or none) may affect voluntary intakes. Although the nutri-

tional characteristics exhibited by autumn-stockpiled tall fescue forage appear to be suitable for developing dairy heifers in the southern Ozark Highlands, additional work is needed that quantifies the voluntary intake of these forages by grazing dairy replacement heifers and other livestock classes, and then identifies appropriate supplementation strategies for maintaining acceptable rates of gain if intakes are inherently depressed.

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