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Relationships Between In Situ Protein Degradability and Grass Developmental Morphology¹

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ABSTRACT

The objective of this research was to determine the relationships between the morphological development and in situ ruminally degradable protein (RDP), ruminally undegradable protein (RUP), and microbial protein of two cool season grasses (intermediate wheatgrass and smooth brome grass) and two warm season grasses (switchgrass and big bluestem). The initial growth of grass tillers grown near Mead, Nebraska was clipped at ground level six times during the 1992 growing season and morphologically classified. Mean stage was calculated. Forage was ground to pass a 2-mm screen and was incubated in ruminally fistulated steers for 16 h. The RUP was adjusted for microbial protein and acid detergent insoluble N. The mean stage of cool season grasses was higher than that of warm season grasses throughout the growing season. The RDP decreased as plant maturity increased for all species. The RUP expressed as a percentage of crude protein for the cool season grasses was lower than that for warm season grasses. The RUP for intermediate wheatgrass, smooth brome grass, and switchgrass remained constant across maturities, but RUP for big bluestem decreased as maturity increased. Microbial augmentation of RUP decreased as crude protein decreased in all species. The RUP corrected for acid detergent insoluble N and microbial protein was relatively constant across plant maturities. The quantification of RUP across a range of plant maturities provided information for incorporating RUP content of forage grasses into the diets of animals.

(**Key words:** grasses, developmental morphology, plant maturity, ruminal escape protein)

Abbreviation key: MP = microbial protein, MSC = mean stage count.

INTRODUCTION

The dietary protein consumed by ruminants is either degraded by proteolysis and deamination in the rumen or escapes to the small intestine. More dietary AA reach the small intestine when protein is protected from ruminal degradation, potentially increasing animal performance (6). Ruminal degradability of forage CP is highly variable among forage species (5, 13, 14) and is affected by stage of maturity (9, 13). The RDP and RUP of forages may be deficient if they are harvested during certain stages of development. Warm season grasses tend to degrade more slowly in the rumen than do cool season grasses (2). Anderson et al. (3) reported that at least 80% of the digestible N of smooth brome grass (*Bromus inermis* Leyss.) was ruminally degradable. Mullahey et al. (13) observed that a mean of 43% of the total CP of switchgrass (*Panicum virgatum* L.) escaped ruminal degradation, and RUP declined as stage of maturity increased. Anatomical differences between species of cool and warm season forages may explain some of the variability in the ruminal degradation of protein (2, 13).

As plants mature, forage quality for ruminants decreases because of an increase in cell-wall concentration, a decrease in cell-wall digestibility, and a decrease in CP. Quantifying the maturity of tiller populations may help to characterize nutrient content throughout the cycle of grass development. Limited information is available on ruminal protein degradability of cool and warm season grasses that are harvested at different stages of morphological development. The objective of this research was to determine the relationships between the morphological develop-

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ment and in situ RDP, RUP, and microbial protein (MP) of intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkw. and D. R. Dewey], smooth bromegrass, switchgrass, and big bluestem (*Andropogon gerardii* Vitman).

MATERIALS AND METHODS

This study was conducted in 1992 at the University of Nebraska Agricultural Research and Development Center near Mead. The grasses were sown as monocultures in 1991 on a Sharpsburg silty clay loam soil (fine, montmorillonitic, mesic, Typic Argiudoll) as a randomized complete block arranged in a split-plot design with three replications. Whole-plot treatments (5 × 5 m) were species with subplot treatments (1.7 × 2.5 m) as harvest dates. Fertilizer (110 kg of N/ha) in the form of ammonium nitrate was applied in late May. Lincoln smooth bromegrass and Slate intermediate wheatgrass were harvested on May 13 and 27, June 10 and 24, and July 8 and 30. Trailblazer switchgrass and Pawnee big bluestem were harvested on May 20, June 3 and 17, July 2 and 14, and August 12.

Tillers used for morphological classification were hand-clipped at ground level from 0.09-m² quadrats randomly located within each whole plot. The maturity of tiller populations was classified using the system described by Moore et al. (12). Mean stage count (MSC) and mean stage weight were determined as estimates of the morphological development of the grass tiller populations for each species at each harvest date. The MSC and mean stage weight were highly correlated ($r^2 = 0.99$). Therefore, MSC was used as the estimate of the morphological development of the grass tiller populations because it was easier to calculate in the field. The whole-plant samples used to analyze DM and CP disappearance of standing green herbage were harvested at ground level from randomly located 0.25-m² quadrats. The samples were oven-dried at 55°C and ground to pass a 2-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA). Plant CP was determined using the Kjeldahl method (4).

In situ DM and CP degradability were estimated for each species at each harvest date using the modified in situ polyester bag technique described by Wilkerson et al. (17). Five-gram samples from each field replication were placed into polyester bags (10 × 20 cm; pore size = $53 \pm 10 \mu\text{m}$) that had been heat-sealed on three sides (Ankom, Fairport, NY). Samples were separated into cool and warm season grasses, randomized into four groups of 18 samples (9 cool season and 9 warm season grass samples), and

placed in a zippered, nylon mesh bag (30 × 45 cm). Three smooth bromegrass standards were added to each sample group to measure variability between steers. Mesh bags were soaked for 30 min in 40°C tap water to hydrate the forage and then were placed in the ventral sac of two ruminally fistulated Angus × Hereford steers (*Bos taurus*) that were fed a mixed diet of smooth bromegrass, switchgrass, and big bluestem hay twice daily for ad libitum intake. The bags were removed following a 16-h incubation period and rinsed in 40°C running tap water with slight agitation until the rinse water was clear and free of ruminal debris (17). Following the primary rinsing stage, individual bags were rinsed in 40°C running tap water to rinse the residue to the bottom of the bag. Bags were dried at 55°C for 72 h and weighed to determine DM loss. Subsamples of the digested forage were analyzed for residual CP using the Kjeldahl method.

The RDP was expressed as a percentage of CP and was calculated by subtracting the sum of RUP and indigestible protein from the initial CP of the forage. The indigestible protein fraction was estimated by ADIN and was subtracted from the RUP. Because the calculated RUP also included residual ruminal MP, the plant contribution to RUP was overestimated. Purine N was isolated and quantified using the technique described by Zinn and Owens (18), which was modified to use the pellet rinsing procedure described by Aharoni and Tagari (1) to determine MP contribution to RUP. Independent purine to N ratios were determined for each species. Microbial contribution to RUP, determined by concentration of purine N, was subtracted from the RUP to adjust for microbial association with forage particles. Adjusted RUP, corrected for ADIN and MP, was calculated using the following equation: adjusted RUP (percentage of CP) = [residual weight × (residual N – ADIN – microbial N)] / (sample weight × sample N) × 100. The RDP, RUP, and ADIN were calculated as a percentage of total plant CP to facilitate uniform comparisons of the CP fractions of the cool and warm season grasses. Additionally, the RUP was calculated as a percentage of DM to provide additional information.

Data were analyzed as a split plot in time; species were whole plots, and harvest dates were subplots using the ANOVA procedure of SAS (15). Treatment means of significant ($P \leq 0.05$) main effects and interactions were compared using Fisher's protected least significant difference at $\alpha = 0.05$ (16). Pearson correlation coefficients between MSC and day of the year, CP, ADIN, MP, RDP, and RUP were calculated independently for each species (15).

RESULTS AND DISCUSSION

Tiller Maturity

The MSC was calculated to quantify numerically the maturity of the tiller population of individual species and should be interpreted as the mean that represents all of the growth stages present in the population (11). A small MSC indicated a less mature tiller population, and a large MSC indicated a more mature tiller population (12). The MSC of the cool and warm season grasses was influenced ($P = 0.0001$) by the interaction of species and harvest date. The MSC for intermediate wheatgrass increased linearly until the final harvest when MSC was reduced because of the appearance of new tillers (Figure 1). Smooth brome grass developed more rapidly than did intermediate wheatgrass early in the growing season, resulting in the higher MSC at harvests 1 and 2 (Figure 1). However, as the growing season progressed, appearance of new vegetative smooth brome grass tillers caused the MSC for smooth brome grass to decline. Intermediate wheatgrass had a larger proportion of tillers reach reproductive maturity than did smooth brome grass, resulting in higher MSC for intermediate wheatgrass late in the season.

The MSC of switchgrass and big bluestem increased linearly throughout the growing season (Figure 1). Switchgrass matured more rapidly and had a larger proportion of tillers reach reproductive maturity than did big bluestem, resulting in higher MSC for switchgrass (Figure 1). Few big bluestem tillers exerted inflorescences prior to the completion

of harvest. The MSC of all species was correlated positively with day of the year (Table 1). The lower temperatures for optimum growth associated with the cool season grasses resulted in a more rapid initiation of growth, elongation, and reproduction, causing a higher MSC than that for the warm season grasses.

Total CP Concentration

Total CP of the cool and warm season grasses was influenced ($P = 0.0002$) by the interaction of species and harvest date. Total CP concentration was higher for cool season grasses than for warm season grasses, especially later in the growing season (Table 2). Total CP concentration of smooth brome grass was higher than that of intermediate wheatgrass at later harvests because of the lower MSC of smooth brome grass later in the growing season. Switchgrass and big bluestem CP followed similar trends as the growing season progressed. The CP of all species decreased as MSC increased, except that the CP of intermediate wheatgrass and smooth brome grass increased between d 134 and 147, and the CP of switchgrass increased between d 140 and 156 (Table 2). The decrease in CP as tiller maturity increased was evident by the negative correlation among the MSC of intermediate wheatgrass, switchgrass, and big bluestem and CP (Table 1). The low correlation with smooth brome grass (-0.147) was likely due to the relatively constant CP concentration of smooth brome grass (Table 2). The decrease in CP as maturity increased was consistent with results of other research (7, 10, 13).

TABLE 1. Pearson correlation coefficients and P values between mean stage count (MSC) and day of the year (DOY), CP, ADIN, microbial protein (MP), RDP, and RUP of intermediate wheatgrass, smooth brome grass, switchgrass, and big bluestem harvested on six dates during 1992 near Mead, Nebraska.

	Cool season grasses				Warm season grasses			
	Intermediate wheatgrass		Smooth brome grass		Switchgrass		Big bluestem	
	r	P	r	P	r	P	r	P
DOY	0.854	0.0001	0.708	0.0010	0.980	0.0001	0.713	0.0009
CP ¹	-0.797	0.0001	-0.147	0.5599	-0.924	0.0001	-0.703	0.0011
ADIN ²	0.756	0.0003	-0.041	0.8723	0.655	0.0032	0.612	0.0069
MP ³	-0.733	0.0005	-0.623	0.0057	-0.820	0.0001	-0.348	0.1568
RDP ²	-0.789	0.0001	-0.124	0.6252	-0.783	0.0001	-0.179	0.4765
RUP ²	0.602	0.0082	0.236	0.3466	0.398	0.1021	-0.562	0.0153
RUP ¹	-0.115	0.6487	0.177	0.4833	-0.749	0.0004	-0.678	0.0020
Observations, no.	18		18		18		18	

¹Calculated as a percentage of total DM.

²Calculated as a percentage of plant CP.

³Calculated as a percentage of RUP.

Indigestible N

The concentration of indigestible N, measured as ADIN, of the cool and warm season grasses was influenced ($P = 0.0001$) by species. The MSC for all species was correlated positively with ADIN, which indicated that ADIN increased as MSC increased (Table 1). The ADIN concentrations of intermediate

wheatgrass, smooth bromegrass, and switchgrass were similar when averaged across maturities (15.6, 16.7, and 17.7% of plant CP, respectively) (Table 2). The ADIN concentration of big bluestem tended to be higher than that of the other species and averaged 28.1% of plant CP (Table 2). Higher concentrations of indigestible N in big bluestem than those in smooth bromegrass and switchgrass were consistent with

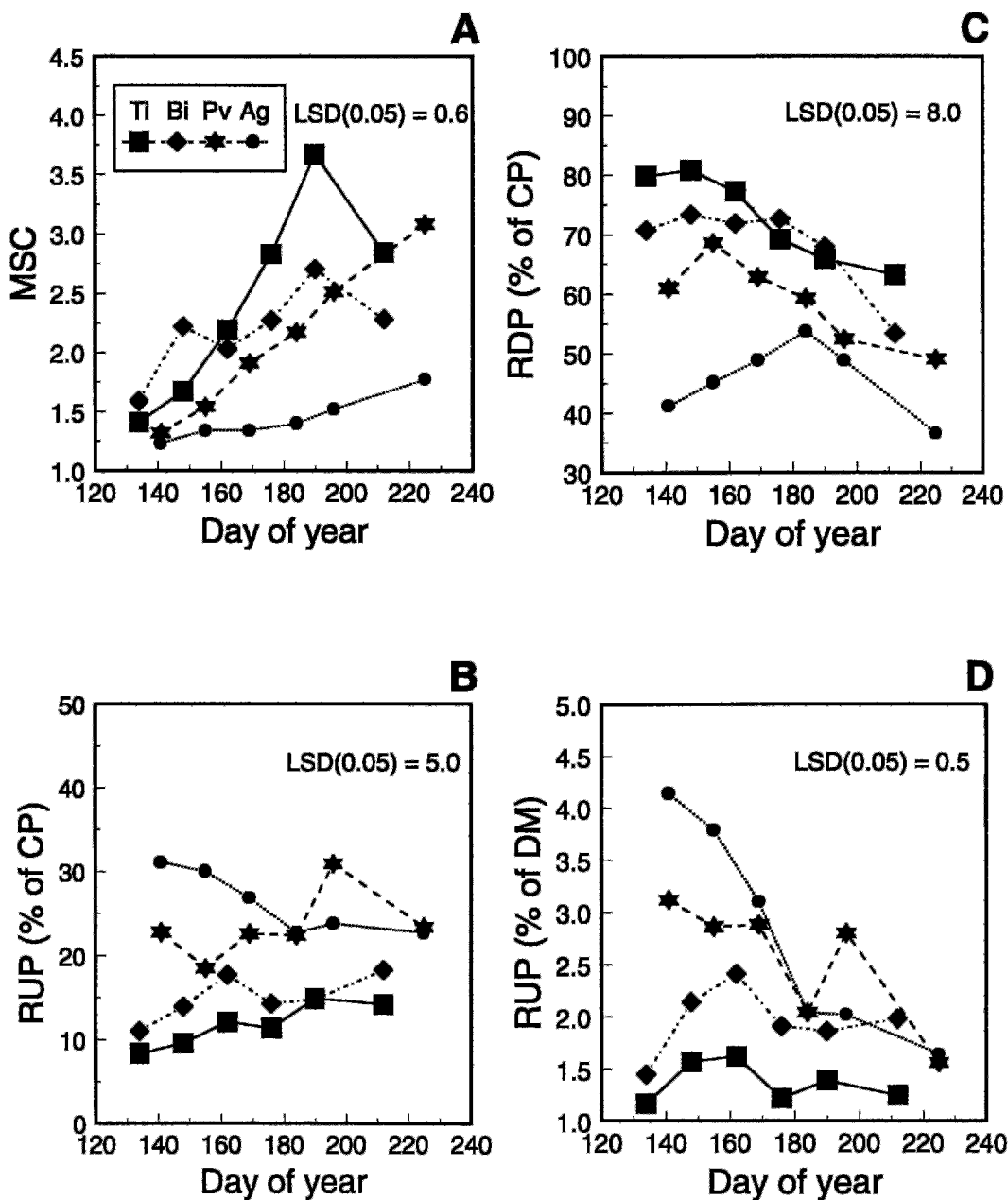


Figure 1. A) Mean stage count (MSC), B) RUP as a percentage of CP, C) RDP as a percentage of CP, and D) RUP as a percentage of DM for intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkw. & D. R. Dewey; Ti), smooth bromegrass (*Bromus inermis* Leyss.; Bi), switchgrass (*Panicum virgatum* L.; Pv) and big bluestem (*Andropogon gerardii* Vitman; Ag) harvested at six dates during the 1992 growing season near Mead, Nebraska. Fisher's protected least significant difference (LSD) at $\alpha = 0.05$ is included.

previous research. Redfearn et al. (14) reported that the percentage of indigestible N in smooth brome-grass and switchgrass was 3 and 15%, respectively, and the percentage of indigestible N in big bluestem was nearly 21%.

MP

The MP concentration, expressed as a percentage of RUP of the cool and warm season grasses following 16 h of in situ ruminal incubation, was influenced ($P = 0.0001$) by the interaction of species and harvest date. The MP of cool and warm season grasses generally decreased as the growing season progressed (Tables 1 and 2). Plant maturity and MP were more highly correlated for intermediate wheatgrass, smooth brome-grass, and switchgrass than for big bluestem (Table 1). The range of MP for intermediate wheatgrass and smooth brome-grass was greater than the range of MP for switchgrass and big bluestem (Table 2). The MP typically decreased as CP decreased, indicating an increased microbial association with higher concentrations of forage CP. The negative correlation between MSC and MP indicated that the association between MP and forage particles decreased as plant maturity increased (Table 1). The reduced concentration of MP might have resulted from higher lignin concentrations associated with advanced maturity, which could physiochemically inhibit microbial activity (2).

RDP

The RDP of cool and warm season grasses was influenced ($P = 0.0051$) by the interaction of species and harvest date. The RDP was similar for intermediate wheatgrass and smooth brome-grass (Figure 1) and was greater than the RDP for switchgrass and big bluestem across maturities (Figure 1). The RDP was correlated negatively with MSC for all species (Table 1). The RDP of big bluestem was lower than that of switchgrass at similar MSC (Figure 1). These results were consistent with Anderson et al. (3) and Mullahey et al. (13), who determined that at least 74% of the digestible protein fraction of smooth brome-grass and 57% of the digestible protein fraction of switchgrass degraded following 12 h of in situ fermentation.

RUP

Forage RUP expressed as a percentage of CP describes the percentage of CP that escaped ruminal degradation and had escape protein value. The RUP,

calculated as a percentage of the CP of cool and warm season grasses and adjusted for MP and ADIN, was influenced ($P = 0.0003$) by the interaction of species and harvest date. The RUP of cool season grasses was typically lower than the RUP of warm season grasses and remained relatively constant across tiller maturities (Figure 1). The RUP of intermediate wheatgrass, smooth brome-grass, and switchgrass was correlated positively with MSC, but the RUP of big bluestem was correlated negatively with MSC (Table 1). The RUP of intermediate wheatgrass ranged from 5 to 15% of total plant CP and increased only slightly as tiller maturity increased (Figure 1). The RUP of smooth brome-grasses ranged from 11 to 18% of total plant CP, varied only slightly across tiller maturities, and was similar to intermediate wheatgrass on common days of the year (Figure 1). The RUP of switchgrass ranged from 23 to 31% of total plant CP (Figure 1). Excluding the d 196 harvest, the RUP of switchgrass tended to decrease as tiller maturity increased (Figure 1). However, switchgrass at d 196 had the highest RUP concentration (Figure 1). The RUP of big bluestem decreased linearly as maturity increased (Figure 1).

Forage RUP expressed as a percentage of DM is the actual amount of protein that escapes ruminal degradation. The RUP calculated as a percentage of DM of cool and warm season grasses, adjusted for MP and ADIN, was influenced ($P = 0.0001$) by the interaction of species and harvest date. The RUP of cool season grasses was typically lower than the RUP of warm season grasses (Figure 1). The RUP of intermediate wheatgrass and smooth brome-grass was poorly correlated with MSC; however, the RUP of switchgrass and big bluestem was highly correlated negatively with MSC (Table 1). The RUP of intermediate wheatgrass ranged from 1.2 to 1.6% of DM and did not differ significantly as tiller maturity increased (Figure 1). The RUP of smooth brome-grass ranged from 1.5 to 2.4% of DM, varied only slightly across tiller maturities, and followed trends that were similar to intermediate wheatgrass on common days of the year (Figure 1). The RUP of switchgrass ranged from 1.6 to 3.1% of DM (Figure 1). Excluding the d 196 harvest, the RUP of switchgrass tended to decrease as tiller maturity increased (Figure 1). The RUP of big bluestem ranged from 1.6 to 4.1% of DM and decreased rapidly as maturity increased (Figure 1).

The RUP values for smooth brome-grass, switchgrass, and big bluestem in this study were lower than those reported by Hafley (8) and Mullahey et al. (13). Hafley (8) reported approximately 42 to 54% of

TABLE 2. The CP, ADIN, and microbial protein (MP) concentration of intermediate wheatgrass, smooth bromegrass, switchgrass, and big bluestem harvested on six dates during 1992 near Mead, Nebraska.

Day of year	Cool season grasses						Day of year	Warm season grasses					
	Intermediate wheatgrass			Smooth bromegrass				Switchgrass			Big bluestem		
	CP	ADIN	MP	CP	ADIN	MP		CP	ADIN	MP	CP	ADIN	MP
	(% of DM)	(% of CP)	(% of RUP)	(% of DM)	(% of CP)	(% of RUP)		(% of DM)	(% of CP)	(% of RUP)	(% of DM)	(% of CP)	(% of RUP)
134	14.0	11.9	16.9	13.5	18.3	13.9	140	13.7	16.2	5.7	13.3	27.8	5.7
147	16.6	9.6	15.4	15.4	12.7	8.5	156	15.4	12.9	8.0	12.6	24.9	5.0
160	13.4	10.7	8.6	13.7	10.4	5.7	171	12.8	14.6	5.8	11.5	24.2	5.4
175	10.7	19.4	8.8	13.5	13.1	6.8	183	9.2	18.3	4.5	9.0	23.5	5.7
195	9.3	19.2	6.5	12.7	17.3	6.5	196	9.0	16.6	2.7	8.5	27.3	5.4
212	8.8	22.6	5.1	10.8	28.3	5.6	224	6.6	27.6	2.6	7.2	40.8	3.4
LSD (0.05)	1.4	NS ¹	2.8	1.4	NS	2.8		1.4	NS	2.8	1.4	NS	2.8

¹P > 0.05.

forage protein escaped ruminal degradation in a mixed stand of big bluestem and switchgrass. Mullahey et al. (13) observed that a mean of 21% of the available N in smooth bromegrass and 51% of the available N in switchgrass escaped ruminal degradation. However, the values reported by Mullahey et al. (13) were calculated on an available N basis, not a total plant N basis, and were not adjusted for MP. Not adjusting for MP likely overestimated RUP as a percentage of total plant CP. Values reported in our study were calculated as a percentage of total CP, and only the residual CP was adjusted for ADIN and MP. Therefore, adjusted RUP of the four species presented in this study represent the combined effect of plant CP, MP, and ADIN concentrations and represent the available RUP presented to the small intestine for potential digestion and absorption in the lower tract.

CONCLUSIONS

Intermediate wheatgrass and smooth bromegrass generally had higher concentrations of RDP and lower concentrations of RUP than did switchgrass and big bluestem, possibly because of differences in the anatomical organization of the tissues of cool and warm season grasses (2). The RDP of intermediate wheatgrass and smooth bromegrass decreased as MSC increased. The RUP of the cool season grasses was typically unaffected by MSC. The RDP of switchgrass and big bluestem was lower than the RDP of intermediate wheatgrass and smooth bromegrass. Consequently, concentrations of RUP as a percentage of total plant CP in switchgrass and big bluestem were nearly two times higher than those in intermediate wheatgrass and smooth bromegrass. However, the differences in RUP of the cool and warm season

grasses were not as large in this study as in previously reported research (8, 13), partially because of adjustments for microbial association with forage particles and because of differences in the calculation of RUP.

Higher concentrations of RUP in the lower tract of ruminants fed warm season grasses might explain the greater than expected performance of livestock when fed warm season grasses, considering the low CP and high NDF concentrations (13). Our study indicated that relationships exist between developmental morphology and RDP, RUP, and MP in perennial forage grasses. However, the relationships between developmental morphology and RDP and RUP were highly variable among species, even within categories of cool and warm season grasses. Therefore, general recommendations concerning the RDP and RUP of forage species should be considered carefully or avoided unless information exists across a variety of maturities within a species. The quantification of RUP in intermediate wheatgrass, smooth bromegrass, switchgrass, and big bluestem across a range of maturities provided additional information for incorporating RUP of perennial forage grasses into ruminant diets. However, the relative economic values of differences in RDP and RUP quantified in this study within both cool and warm season grasses need to be determined in lactation and grazing trials before management and diet recommendations are developed for use by producers.

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