

Harvest Timing Effects on Estimates of Rumen Degradable Protein from Alfalfa Forages

W. K. Coblenz,^{*} G. E. Brink, N. P. Martin, and D. J. Undersander

ABSTRACT

Alfalfa (*Medicago sativa* L.) proteins ingested by dairy cows typically degrade at rapid rates and exhibit extensive ruminal degradability. Although the effects of conservation method (hay or silage) on these characteristics have been evaluated extensively, agronomic factors, such as harvest timing, have not. Our objective was to quantify rumen degradable protein (RDP) for 'Affinity' alfalfa harvested over a range of ages (0, 5, 10, 15, and 20 d following Stage 2) within each of four harvest periods (spring, early and late summer, and fall). For 2004, there were no interactions ($P \geq 0.372$) between harvest period and days within harvest period for any protein component. Crude protein (CP), neutral-detergent soluble CP (NDSCP; g kg^{-1} dry matter [DM]), and RDP (g kg^{-1} DM) declined in a quadratic ($P \leq 0.026$) relationship with days following Stage 2. A quadratic ($P = 0.002$) pattern also was observed for rumen undegradable protein (RUP), but the overall range was small (60.4–66.5 g kg^{-1} DM). On a CP basis, RDP declined linearly ($P < 0.001$) from 720 to 659 g kg^{-1} CP during 2004. For 2005, there were interactions ($P \leq 0.020$) of harvest period and days within period for all protein-related response variables, but trends over time within each harvest period generally were similar to those observed in 2004. Overall, RDP declined as alfalfa plants aged within harvest period, but these responses were due primarily to reduced concentrations of CP within the cell-soluble fraction.

W.K. Coblenz, USDA-ARS, U.S. Dairy Forage Research Center, 8396 Yellowstone Dr., Marshfield, WI 54449; G.E. Brink and N.P. Martin, USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI 53706; D.J. Undersander, Dep. of Agronomy, University of Wisconsin, Madison, WI 53706. Mention of trade names or commercial products is solely for the purpose of providing specific information about scientific procedures and/or subsequent evaluation of results, and does not imply any recommendation or endorsement by the USDA. Received 1 June 2007. ^{*}Corresponding author (wayne.coblenz@ars.usda.gov).

Abbreviations: CP, crude protein; DM, dry matter; ES, early-summer harvest period; FA, fall harvest period; GDD, growing degree days; LS, late-summer harvest period; NDF, neutral-detergent fiber; NDICP, neutral-detergent insoluble CP; NDSCP, neutral-detergent soluble CP; RDP, rumen degradable protein; RUP, rumen undegradable protein; SP, spring harvest period.

MANY CURRENT NUTRITIONAL MODELS for ruminants require knowledge of the concentrations of rumen degradable protein (RDP) and rumen undegradable protein (RUP) within forages (Sniffen et al., 1992; National Research Council, 1996, 2001). This concept is based on the premise that the protein requirements of ruminants are met by both RUP and microbial proteins synthesized within the rumen. Broderick (1985) has suggested that alfalfa (*Medicago sativa* L.) proteins are degraded rapidly in the rumen, thereby causing inefficient utilization by lactating dairy cows. In addition to costs associated with this inefficiency, excess dietary N is voided from the cow via the urine as urea, thereby increasing potential negative impacts on the environment.

The effects of harvest and storage management on the nutritive value of hays and silages are well documented (Rotz and Muck, 1994). Broderick et al. (1992) reported that harvest and

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storage as hay reduced protein degradation rate from 0.171 to 0.075 h⁻¹, and increased RUP from 240 to 397 g kg⁻¹ crude protein (CP), relative to freeze-dried standing forage. Several studies have shown that ruminal degradation of forage proteins from alfalfa hay can be limited by externally applied heat treatment (Broderick et al., 1993; Yang et al., 1993) or by spontaneous heating during storage (Coblentz et al., 1997). However, these increases in RUP can be complicated by concurrent increases in acid detergent insoluble CP (Broderick et al., 1993; Yang et al., 1993; Coblentz et al., 1996), which is assumed to have low bioavailability (Licitra et al., 1996). Accumulation of acid-detergent insoluble CP also occurs in response to spontaneous heating during ensiling, a process that is common in drier silages (Rotz and Muck, 1994).

While these research initiatives have focused heavily on conservation as hay or silage, effects of agronomic management factors, such as harvest timing, are less clear. Using in situ methodology, Hoffman et al. (1993) found that RDP (g kg⁻¹ CP) decreased with plant maturity for alfalfa, red clover (*Trifolium pratense* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) harvested at the late vegetative, late bud, and midbloom stages of growth; however, this response was largely the result of changing proportions of soluble, slowly degraded, and unavailable fractions within the total pool of CP rather than a less rapid degradation rate for more mature forages. Cassida et al. (2000) reported some increases in RUP (g kg⁻¹ dry matter [DM]) with plant maturity for alfalfa, red clover, and birdsfoot trefoil forages; however, these responses were sharper when RUP was reported on a gram per kilogram CP basis. In contrast, Broderick et al. (1992) used an in vitro technique that prevents the uptake of protein degradation products by microbes for subsequent protein synthesis (Broderick, 1994) and found no relationship between plant maturity and either degradation rate or RUP (g kg⁻¹ CP) for 89 alfalfa forages harvested over a range of maturities, cuttings, and years. Therefore, the relationship between harvest timing and/or plant maturity and subsequent estimates of RUP or RDP remains unclear, and may be confounded strongly by climatic conditions during growth (Griffin et al., 1994; Cassida et al., 2000).

Currently, the in situ procedure (Vanzant et al., 1998), corrected for microbial contaminant N, is the most common method used for evaluating relative proportions of RDP and RUP in ruminant feedstuffs; however, in vitro procedures that utilize semipurified proteolytic enzymes have been developed as routine laboratory techniques for the estimation of these protein fractions in forages (Krishnamoorthy et al., 1983; Licitra et al., 1998; Coblentz et al., 1999). Generally, single-endpoint enzymatic techniques can more easily accommodate the sample numbers generated from plot-type studies than full time-course kinetic evaluations by in situ methodologies. Our primary objec-

tive for this study was to utilize a preparation of *Streptomyces griseus* protease to assess the effects of harvest period and days within harvest period on concentrations of RDP and RUP for alfalfa forages harvested at Prairie du Sac, WI, during 2004 and 2005.

MATERIALS AND METHODS

Plot Management

During August 2003, a Richwood silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll) soil, located near Prairie du Sac, WI, was amended to meet soil-test recommendations for P, K, and pH, and 'Affinity' alfalfa was then drilled into a prepared seedbed at a rate of 22 kg ha⁻¹. Four 7.6 by 6.1 m plots were established in each of four field blocks. After establishment, soil tests were taken annually, and amendments were applied as needed to meet soil test recommendations of the University of Wisconsin Cooperative Extension Service. During the course of the study, potato leafhoppers (*Empoasca fabae* Harris) were controlled as needed with applications of lambda-cyhalothrin {[1 α (S*),3 α (Z)]-(\pm)-cyano-(3-phenoxyphenyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate} at a rate of 0.024 kg a.i. ha⁻¹.

Beginning in the spring of 2004, the four plots within each block were assigned randomly to one of four harvest periods (spring [SP], early summer [ES], late summer [LS], and fall [FA]). For the SP period, one 1.5 by 6.1 m strip from each plot was harvested when plant maturity reached Stage 2 (Kalu and Fick, 1981). At this time, designated as Day 0, stem length was >0.30 m, but no buds, flowers, or seedpods were visible. Subsequently, additional 1.5 by 6.1 m strips were assigned randomly throughout the plot, and harvested at 5-d intervals for a total of five strips per plot (0, 5, 10, 15, and 20 d). Although there was some variability between harvest periods, sampling dates were structured such that Day 10 generally coincided with a one-tenth bloom stage of development. In addition, this 20-d harvest window within each harvest period represented a realistic range of time over which most producers would typically harvest alfalfa in Wisconsin. While harvesting during the SP period, plots designated for the ES, LS, and FA harvest periods were clipped at 1/10 bloom, but no samples or data were collected. These harvest procedures were then repeated later during the growing season as plots assigned to the ES, LS, and FA harvest periods reached Stage 2, as defined previously. All sampling dates and growing degree days (GDD) accumulated within each harvest period during 2004 and 2005 are reported in Table 1. Growing degree days were calculated daily by subtracting 5°C from the average of the maximum and minimum temperatures for that day, and then summing over days within each harvest period.

Some discussion of postsampling management also is warranted. Following data collection from the 0, 5, 10, 15, and 20-d strips of the SP plot, regrowth from all SP strips was allowed to reach a minimum of one-tenth bloom before the next (ES) harvest. At that time, no data were taken, and all harvested forage from the SP plot was discarded. For each subsequent harvest period (LS and FA), the entire SP plot was harvested at one-tenth bloom, but no data were recorded. Identical postsampling procedures were used for plots designated for data

Table 1. Harvest dates and growing degree days (GDD) within spring (SP), early-summer (ES), late-summer (LS), and fall (FA) harvest periods for 2004 and 2005.

| Harvest period | Days | 2004 | | 2005 | |
|----------------|------|----------|------------------|----------|-----|
| | | Date | GDD [†] | Date | GDD |
| SP | 0 | 24 May | 347 | 16 May | 264 |
| | 5 | 2 June | 433 | 21 May | 310 |
| | 10 | 7 June | 489 | 25 May | 350 |
| | 15 | 11 June | 564 | 31 May | 406 |
| | 20 | 16 June | 633 | 3 June | 444 |
| ES | 0 | 28 June | 282 | 17 June | 336 |
| | 5 | 2 July | 338 | 22 June | 408 |
| | 10 | 7 July | 418 | 27 June | 506 |
| | 15 | 12 July | 479 | 1 July | 584 |
| | 20 | 16 July | 547 | 6 July | 656 |
| LS | 0 | 4 Aug. | 435 | 25 July | 499 |
| | 5 | 9 Aug. | 502 | 29 July | 564 |
| | 10 | 13 Aug. | 550 | 2 Aug. | 631 |
| | 15 | 18 Aug. | 598 | 8 Aug. | 741 |
| | 20 | 23 Aug. | 648 | 11 Aug. | 799 |
| FA | 0 | 8 Sept. | 336 | 1 Sept. | 473 |
| | 5 | 13 Sept. | 395 | 6 Sept. | 542 |
| | 10 | 17 Sept. | 459 | 9 Sept. | 596 |
| | 15 | 22 Sept. | 519 | 14 Sept. | 687 |
| | 20 | 27 Sept. | 576 | 20 Sept. | 761 |

[†]Growing degree days (GDD) were calculated daily by subtracting 5°C from the average of the maximum and minimum temperatures for that day, and then summing over days within each harvest period.

collection during the ES or LS harvest periods. These procedures were designed to minimize any carryover effects from previous harvests or years, and maximize the statistical independence of each harvest period. The study was conducted over 2 yr (2004 and 2005); therefore, a total of eight harvest periods were included in the experiment. Within block, plot assignments for individual harvest periods (SP, ES, LS, and FA) and sampling dates within harvest period were maintained without additional randomization between years.

Sample Preparation and Analysis

All harvested alfalfa forages were dried for 48 h under forced air at 50°C, and ground subsequently through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) equipped with a 1-mm screen. Concentrations of CP in each sample were quantified by a macro-Kjeldahl technique (Association of Official Analytical Chemists, 1998), where CP was calculated by multiplying the percentage of N in each forage sample by 6.25. Samples were then analyzed for neutral-detergent fiber (NDF) using the batch procedures outlined by ANKOM Technology Corporation (Fairport, NY). Sodium sulfite and heat-stable α -amylase were not included in the NDF solution. Following incubation in neutral detergent, neutral-detergent insoluble CP (NDICP) was determined by analyzing insoluble fibrous residues for concentrations of CP using the same macro-Kjeldahl technique described for quantification of whole-plant concentrations of CP. Concentrations of NDICP were reported as both proportions of whole-plant DM and CP. Neutral detergent soluble CP

was calculated as CP – NDICP, where NDICP was expressed as a proportion of whole-plant DM.

In Vitro Incubation in Prepared Protease Solution

The in vitro protease procedures used in this study were similar to those described by Krishnamoorthy et al. (1983), Licitra et al. (1998), and Coblenz et al. (1999). *Streptomyces griseus* protease (P-5147; Sigma Chemical Co., St. Louis, MO) contained 4.5 enzyme activity units per milligram of solid, where one activity unit of enzyme was able to hydrolyze casein to produce color equivalent to 1.0 μ mol (181 μ g) of tyrosine per minute at pH 7.5 and 37°C. Sample size for all incubations was set on the basis of a common N content (15 mg) within each incubation flask; therefore, the actual sample weight was adjusted for CP concentration, and varied somewhat across forages. Each forage sample was incubated in a water bath for 1 h at 39°C in 40 mL (pH 8.0) of borate-phosphate buffer (Krishnamoorthy et al., 1983). Following the 1-h buffer incubation, 10 mL of prepared protease solution containing 0.33 activity units mL⁻¹ of *S. griseus* protease was added to each flask, yielding a final enzyme activity concentration of 0.066 activity units mL⁻¹ in the incubation medium. Flasks were covered with aluminum foil, swirled daily, and incubated for 48 h at 39°C. One milliliter of sodium azide (1%, w/v) was added to each incubation flask as an antimicrobial agent. Following incubation, samples were immediately filtered through preweighed (dry basis) Whatman no. 541 filter paper (Whatman International Ltd., Maidstone, UK). Residues were washed with approximately 400 mL of deionized water (20°C), and dried in a gravity convection oven at 100°C; these residues were then analyzed for CP by the macro-Kjeldahl technique described previously. Single time-point estimates of RDP were calculated as RUP (g kg⁻¹ DM) = (g residual CP/g initial DM) \times 1000, and RDP (g kg⁻¹ DM) = CP – RUP. Estimates of RDP also were expressed on the basis of total plant CP; calculations were made by RDP (g kg⁻¹ CP) = [RDP (g kg⁻¹ DM)/CP] \times 1000. Incubation flasks containing each forage sample were evaluated by the *S. griseus* protease procedure in each of two separate runs. Values from each run were averaged to yield the final RUP and RDP values for each forage replicate.

Statistical Analysis

Originally, year was included within the statistical analysis as a sub-subplot term. However, there were numerous interactions ($P < 0.05$) of year with other treatment effects; therefore, year was dropped from the model, and each year was evaluated independently. This analytical approach precludes evaluation of certain carryover effects of treatment; among these, the most important are comparisons across years of plots with the same combination of harvest period and days within period. However, given the emphasis on independence across both harvests and years that was built intentionally into the experimental design, it is far more likely that differences between years can be attributed to climatic variability, and that climatic effects would dwarf any potential carryover effects of treatment.

Within year, data were analyzed by PROC GLM of SAS (SAS Institute, 1990) as a split-plot experiment with harvest

periods (SP, ES, LS, and FA) designated as the whole-plot term, and time from Stage 2 (0, 5, 10, 15, and 20 d) as the subplot term. Whole plots were arranged in a randomized complete block design with four replications, and were tested for significance with the harvest period \times block mean square as the error term. The subplot term (days from Stage 2) and the interaction of main effects (harvest period \times days) were tested for significance with the residual error mean square. For 2004, most response variables exhibited no interaction ($P > 0.05$) between harvest period and days within harvest period; therefore, main effect means for harvest period were separated with the PDIFF option of SAS (SAS Institute, 1990). Single degree-of-freedom orthogonal contrasts were used to test for linear, quadratic, cubic, and quartic effects of days from Stage 2. Contrasting responses were observed for 2005. Interactions ($P < 0.05$) of harvest period and days were observed for most response variables; therefore, single degree-of-freedom orthogonal contrasts were used to test for linear, quadratic, cubic, and quartic effects of days from Stage 2 within each harvest period. Correlation analysis relating all response variables and GDD was conducted by PROC CORR procedures of SAS (SAS Institute, 1990). In all cases, significance was declared at $P \leq 0.05$, unless otherwise indicated.

RESULTS

Precipitation and Temperature

Generally, 2004 and 2005 could be described as wet and dry years, respectively (Table 2). From April through October 2004, precipitation exceeded the 30-yr norm (NOAA, 2002) by 150 mm, and was greater than normal during every month except April and September. During the months of most active plant growth (May, June, July, and August), the cumulative precipitation surplus totaled 247 mm, and was coupled with respective monthly mean temperatures that were cooler than normal by 1.4, 1.9, 1.8, and 3.0°C. In contrast, there was a 193-mm cumulative precipitation deficit from April through October 2005; during this time period, precipitation exceeded the 30-yr norm in only May (4 mm) and July (41 mm). In addition, mean monthly temperatures exceeded the 30-yr norm by 0.1 to 2.4°C in all months except May.

2004

Neutral-Detergent Fiber

For NDF during 2004, there was an interaction ($P = 0.001$; Table 3) between harvest period and days. Within the SP, ES, LS, and FA harvest periods (Table 4), concentrations of NDF increased numerically over the 20 d that followed Stage 2 (91, 78, 59, and 47 g kg⁻¹, respectively). For the SP, ES, and LS harvest periods, NDF increased consistently over each 5-d increment, exhibiting linear ($P < 0.001$)

Table 2. Monthly average temperature and cumulative precipitation at Prairie du Sac, WI, from January 2004 through December 2005.

| Month | Precipitation | | | Temperature [†] | | |
|-------------------|---------------|------|-------------------------|--------------------------|------|-------------------------|
| | 2004 | 2005 | 30-yr norm [‡] | 2004 | 2005 | 30-yr norm [‡] |
| | mm | | | °C | | |
| Jan. | 15 | 53 | 26 | -9.4 | -8.3 | -8.9 |
| Feb. | 19 | 27 | 27 | -5.3 | -1.9 | -5.9 |
| Mar. | 78 | 18 | 50 | 3.2 | -0.7 | 0.3 |
| Apr. | 51 | 19 | 80 | 8.0 | 9.7 | 7.8 |
| May | 219 | 82 | 78 | 13.4 | 12.5 | 14.8 |
| June | 175 | 22 | 100 | 18.0 | 22.3 | 19.9 |
| July | 113 | 138 | 97 | 20.4 | 22.3 | 22.2 |
| Aug. | 124 | 49 | 109 | 17.8 | 20.9 | 20.8 |
| Sept. | 11 | 78 | 81 | 17.7 | 18.3 | 15.9 |
| Oct. | 54 | 20 | 56 | 9.7 | 10.4 | 9.4 |
| Nov. | 40 | 66 | 52 | 3.9 | 2.5 | 1.3 |
| Dec. | 39 | 13 | 31 | -4.4 | -6.8 | -5.6 |
| Total/annual mean | 939 | 586 | 788 | 7.8 | 8.4 | 7.7 |

[†]Temperature data from Prairie du Sac were incomplete. Monthly mean temperatures for 2004 and 2005 were obtained from Baraboo, WI, which is 26 km from Prairie du Sac.

[‡]NOAA (2002).

effects of time in each case. A quartic effect ($P = 0.012$) also was observed for the SP harvest period. In contrast, a 30 g kg⁻¹ DM increase between Days 0 and 5 was observed for the FA harvest period, which was followed by essentially no change thereafter (range = 402 to 408 g kg⁻¹ DM), and an overall quadratic ($P = 0.004$) effect of time. Although concentrations of NDF increased numerically with each 5-d increment for all harvest periods, this lack of response at 10, 15, and 20 d for the FA harvest period likely created the interaction of main effects that was not observed ($P > 0.05$) for any other response variable during 2004.

Harvest Period Effects

Concentrations of CP differed ($P < 0.05$) across harvest periods, ranging from 193 to 230 g kg⁻¹ DM (Table 5).

Table 3. Probabilities ($P > F$) for main effects and their interaction for alfalfa forages harvested during 2004 and 2005 at Prairie du Sac, WI.[†]

| Effect | NDF | CP | NDSCP | NDICP | NDICP | RUP | RDP | RDP |
|---------------------|--------|--------|--------|-----------------|-------|--------|--------|--------|
| | | | | | | | | |
| 2004 | | | | | | | | |
| Harvest period (HP) | <0.001 | <0.001 | <0.001 | NS [‡] | NS | NS | <0.001 | 0.004 |
| Days | <0.001 | <0.001 | <0.001 | NS | 0.016 | 0.017 | <0.001 | <0.001 |
| HP \times days | 0.001 | NS | NS | NS | NS | NS | NS | NS |
| 2005 | | | | | | | | |
| HP | 0.028 | NS | NS | 0.035 | 0.021 | <0.001 | 0.001 | <0.001 |
| Days | <0.001 | <0.001 | <0.001 | 0.033 | NS | <0.001 | <0.001 | <0.001 |
| HP \times days | NS | <0.001 | <0.001 | 0.020 | 0.015 | 0.014 | <0.001 | 0.001 |

[†]NDF, neutral-detergent fiber; CP, crude protein; NDSCP, neutral-detergent soluble CP; NDICP, neutral-detergent insoluble CP; RUP, rumen undegradable protein; RDP, rumen degradable protein; DM, dry matter.

[‡]Not significant, $P > 0.05$.

Table 4. Concentrations of neutral-detergent fiber (NDF) for alfalfa forages as affected by days from Stage 2 (Kalu and Fick, 1981). For 2004, there was a harvest period \times days interaction ($P = 0.001$); therefore means are presented for individual spring (SP), early-summer (ES), late-summer (LS), and fall (FA) harvest periods. For 2005, no interaction was found ($P > 0.05$), and results are averaged over all harvest periods.

| Days from Stage 2 | 2004 | | | | 2005 |
|------------------------|------------------------------------|--------|--------|--------|--------|
| | SP | ES | LS | FA | |
| d | g kg ⁻¹ DM [†] | | | | |
| 0 | 394 | 384 | 378 | 361 | 360 |
| 5 | 406 | 396 | 401 | 391 | 392 |
| 10 | 444 | 434 | 403 | 402 | 400 |
| 15 | 449 | 440 | 420 | 406 | 421 |
| 20 | 485 | 462 | 437 | 408 | 449 |
| SEM [‡] | 5.0 | 5.9 | 7.7 | 4.9 | 7.3 |
| Contrasts [§] | $P > F$ | | | | |
| Linear | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Quadratic | NS [¶] | NS | NS | 0.004 | NS |
| Cubic | NS | NS | NS | NS | NS |
| Quartic | 0.012 | NS | NS | NS | NS |

[†]DM, dry matter.

[‡]Standard error of the mean.

[§]Linear, quadratic, cubic, and quartic effects of days within harvest period.

[¶]Not significant, $P > 0.05$.

Table 5. Concentrations of protein components for alfalfa forages harvested from spring (SP), early-summer (ES), late-summer (LS), and fall (FA) harvest periods at 0, 5, 10, 15, and 20 d after reaching Stage 2 (Kalu and Fick, 1981) during 2004 at Prairie du Sac, WI.[†]

| Effect | CP | NDSCP | NDICP | NDICP | RUP | RDP | RDP |
|------------------------|-----------------------|--------|-----------------------|-------|-----------------------|--------|-----------------------|
| | g kg ⁻¹ DM | | g kg ⁻¹ CP | | g kg ⁻¹ DM | | g kg ⁻¹ CP |
| Harvest period | | | | | | | |
| SP | 204c [‡] | 176c | 27.7 | 138 | 68.3a | 135c | 663b |
| ES | 193d | 166d | 27.1 | 141 | 62.5ab | 130c | 674b |
| LS | 213b | 187b | 26.7 | 126 | 58.3b | 155b | 725a |
| FA | 230a | 201a | 28.6 | 124 | 65.0ab | 165a | 716a |
| SEM [§] | 2.3 | 2.4 | 1.22 | 5.9 | 2.41 | 2.1 | 10.1 |
| Days | | | | | | | |
| 0 | 238 | 209 | 29.0 | 122 | 66.5 | 172 | 720 |
| 5 | 216 | 189 | 27.2 | 126 | 63.8 | 152 | 703 |
| 10 | 206 | 180 | 26.7 | 130 | 60.4 | 146 | 706 |
| 15 | 196 | 170 | 26.3 | 134 | 61.8 | 134 | 684 |
| 20 | 192 | 164 | 28.3 | 148 | 65.3 | 127 | 659 |
| SEM | 2.0 | 2.2 | 1.15 | 5.5 | 1.37 | 2.2 | 6.5 |
| Contrasts [¶] | $P > F$ | | | | | | |
| Linear | <0.001 | <0.001 | NS [#] | 0.001 | NS | <0.001 | <0.001 |
| Quadratic | <0.001 | 0.001 | NS | NS | 0.002 | 0.026 | NS |
| Cubic | NS | NS | NS | NS | NS | NS | NS |
| Quartic | NS | NS | NS | NS | NS | NS | NS |

[†]CP, crude protein; NDSCP, neutral-detergent soluble CP; NDICP, neutral-detergent insoluble CP; RUP, rumen undegradable protein; RDP, rumen degradable protein; DM, dry matter.

[‡]Harvest period means within a column that do not have common following letters differ ($P \leq 0.05$).

[§]Standard error of the mean.

[¶]Linear, quadratic, cubic, and quartic effects of days within harvest period.

[#]Not significant, $P > 0.05$.

The greatest ($P < 0.05$) concentration of CP was observed for the FA harvest period, while the smallest ($P < 0.05$) occurred for the ES harvest period. Other harvest periods were intermediate, but differed ($P < 0.05$) from each other. Concentrations of NDSCP ranged from 166 to 201 g kg⁻¹ DM, and paralleled responses for CP. Protein associated with the cell wall matrix (NDICP) did not differ ($P > 0.05$) across harvest periods, regardless of whether it was expressed on a DM (overall mean = 27.5 g kg⁻¹ DM) or CP (overall mean = 132 g kg⁻¹ CP) basis. Although RUP differed ($P < 0.05$) across harvest periods, the magnitude of these differences was relatively small (overall range = 10 g kg⁻¹ DM) with the maximum value from the SP harvest period (68.3 g kg⁻¹ DM) differing ($P < 0.05$) only from the minimum, which was observed for the LS harvest period (58.3 g kg⁻¹ DM). Other harvest periods were numerically intermediate, and did not differ ($P > 0.05$) from either extreme. Because differences across harvest periods for RUP were relatively small, RDP (g kg⁻¹ DM) varied ($P < 0.05$) largely with concentrations of CP. For example, the minimum and maximum concentrations of RDP (130 and 165 g kg⁻¹ DM) were observed for the ES and FA harvest periods, respectively, and these responses also coincided with the greatest and smallest pools of total CP for individual harvest periods. When RDP was expressed on a CP basis, concentrations were greatest ($P < 0.05$) for the FA and LS harvest periods (mean = 721 g kg⁻¹ CP), which differed significantly ($P < 0.05$) from the SP and ES harvests (mean = 669 g kg⁻¹ CP).

Day Effects

Both CP and NDSCP declined quadratically ($P \leq 0.001$) with days from Stage 2 (Table 5). As expected, CP was greatest when plants had just reached Stage 2 (238 g kg⁻¹ DM), and then declined by 19% between Days 0 and 20. Similarly, the pool of CP soluble in neutral detergent (NDSCP) declined by 22% over the same time interval, ranging from 209 g kg⁻¹ DM on Day 0 down to 164 g kg⁻¹ DM on Day 20. Concentrations of NDICP did not differ ($P > 0.05$) between Days 0 and 20, exhibiting an overall mean of 27.5 g kg⁻¹ DM during this time interval. When expressed on a CP basis, NDICP increased in a linear ($P = 0.001$) pattern that can largely be explained on the basis of a stable NDICP pool, and CP concentrations that declined as plants aged within harvest period. Although RUP exhibited a strong, quadratic ($P = 0.002$) relationship with time, the practical value of this response is probably limited. Means for specific days varied narrowly (overall range = 60.4 to 66.5 g kg⁻¹

DM), and the estimates of RUP for Days 0 and 20 varied by only 1.2 g kg⁻¹ DM. Because concentrations of RUP remained relatively consistent between Days 0 and 20, RDP (g kg⁻¹ DM) declined quadratically ($P = 0.026$) over the same time period, which can be associated specifically with concomitant shrinking pools of total CP and NDSCP. By Day 20, RDP declined by 26% relative to the estimate on Day 0. Expressed as a proportion of CP, RDP declined linearly ($P < 0.001$) with days from Stage 2, exhibiting a maximum of 720 g kg⁻¹ CP on Day 0 and a minimum of 659 g kg⁻¹ CP on Day 20.

2005

Neutral-Detergent Fiber

Unlike all other response variables, there was no harvest period \times days within harvest period interaction for NDF during 2005 ($P > 0.05$; Table 3); however, main effects of harvest period ($P = 0.028$) and days ($P < 0.001$) were significant. Concentrations of NDF were greater ($P < 0.05$) for the FA and SP harvest periods (417 and 407 g kg⁻¹ DM, respectively) than observed for the LS period (389 g kg⁻¹ DM). The ES period was numerically intermediate (405 g kg⁻¹ DM), but did not differ ($P > 0.05$) from either extreme (data not shown). Averaged over all harvest periods, NDF increased linearly ($P < 0.001$; Table 4) with days from Stage 2, ranging from 360 to 449 g kg⁻¹ DM between Days 0 and 20, respectively, or a daily increase of about 4.5 g kg⁻¹ DM.

Spring Harvest Period

For 2005, there was a harvest period \times days within harvest period interaction for all protein components ($P \leq 0.020$; Table 3); therefore, protein-related data for 2005 are presented and discussed by harvest period. For the SP harvest period (Table 6), CP declined linearly ($P < 0.001$) from 245 to 171 g kg⁻¹ DM between Days 0 and 20, respectively. Concentrations of NDSCP declined with quartic ($P = 0.026$) and strong linear ($P < 0.001$) effects over the 20-d sampling period; the concentration observed on Day 20 (144 g kg⁻¹ DM) represented only 67% of that observed on Day 0. In contrast, concentrations of both NDICP (g kg⁻¹ DM) and NDICP (g kg⁻¹ CP) exhibited no relationship ($P > 0.05$) with time. Concentrations of RUP exhibited quartic ($P = 0.025$) and linear ($P = 0.022$) changes over time, but the biological relevance of these effects is questionable. The total range of RUP estimates (44.7 to 54.8 g kg⁻¹ DM) across dates was relatively small, and any biological or physiological explanation for the quartic effect of time would be tedious, at best.

Concentrations of RDP (g kg⁻¹ DM) declined over time, but exhibited complex

quartic ($P = 0.017$), cubic ($P = 0.030$), and linear ($P < 0.001$) effects; however, consistent with observations for CP and NDSCP, the concentration of RDP (g kg⁻¹ DM) on Day 20 represented only 66% of that observed on Day 0. Expressed on a CP basis, RDP (g kg⁻¹ CP) also declined over time; however this response also was somewhat erratic, exhibiting both quartic ($P = 0.008$) and linear ($P = 0.004$) effects.

Early-Summer Harvest Period

Concentrations of CP, NDSCP, and RDP all declined with both cubic ($P \leq 0.019$) and linear ($P \leq 0.004$) effects of time (Table 7). Concentrations of NDICP (g kg⁻¹ DM or CP) and RUP (g kg⁻¹ DM) also all declined linearly ($P \leq 0.007$) over time; however, total changes in the NDICP (13.1 g kg⁻¹ DM) and RUP (8.2 g kg⁻¹ DM) pools between Days 0 and 20 were considerably smaller than observed for NDSCP (30 g kg⁻¹ DM) and RDP (35 g kg⁻¹ DM), thereby indicating that CP fractions associated with the cell wall have relatively stable relationships with plant age within harvest period. When RDP was expressed on a CP basis, there was a cubic ($P = 0.007$) effect of time; however, the estimates for Days 0 and 20 varied by only 22 g kg⁻¹ CP, and responses were substantially more erratic than those exhibited during other harvest periods.

Late-Summer Harvest Period

As observed for the previous (ES) harvest period, CP, NDSCP, and RDP (g kg⁻¹ DM) all declined over time with strong linear ($P < 0.001$) effects (Table 8). A quartic ($P = 0.015$) effect also was detected for CP. For these three

Table 6. Concentrations of protein components for alfalfa forages harvested from a spring harvest period at 0, 5, 10, 15, and 20 d after reaching Stage 2 (Kalu and Fick, 1981) during 2005 at Prairie du Sac, WI.[†]

| Days from Stage 2 | CP | NDSCP | NDICP | NDICP | RUP | RDP | RDP |
|------------------------|-----------------------|--------|-----------------|-----------------------|---------|-----------------------|-------|
| d | g kg ⁻¹ DM | | | g kg ⁻¹ CP | | g kg ⁻¹ CP | |
| 0 | 245 | 215 | 30.4 | 123 | 54.8 | 191 | 777 |
| 5 | 239 | 218 | 21.5 | 90 | 45.9 | 193 | 808 |
| 10 | 206 | 179 | 27.1 | 130 | 53.2 | 153 | 743 |
| 15 | 188 | 168 | 20.5 | 109 | 45.4 | 143 | 758 |
| 20 | 171 | 144 | 26.6 | 157 | 44.7 | 126 | 739 |
| SEM [‡] | 5.2 | 5.0 | 4.59 | 20.3 | 2.48 | 4.7 | 11.0 |
| Contrasts [§] | | | | | $P > F$ | | |
| Linear | <0.001 | <0.001 | NS [¶] | NS | 0.022 | <0.001 | 0.004 |
| Quadratic | NS | NS | NS | NS | NS | NS | NS |
| Cubic | NS | NS | NS | NS | NS | 0.030 | NS |
| Quartic | NS | 0.026 | NS | NS | 0.025 | 0.017 | 0.008 |

[†]CP, crude protein; NDSCP, neutral-detergent soluble CP; NDICP, neutral-detergent insoluble CP; RUP, rumen undegradable protein; RDP, rumen degradable protein; DM, dry matter.

[‡]Standard error of the mean.

[§]Linear, quadratic, cubic, and quartic effects of days within harvest period.

[¶]Not significant, $P > 0.05$.

Table 7. Concentrations of protein components for alfalfa forages harvested at 0, 5, 10, 15, and 20 d after reaching Stage 2 (Kalu and Fick, 1981) during 2005 at Prairie du Sac, WI.†

| Days from stage 2 | CP | NDSCP | NDICP | NDICP | RUP | RDP | RDP |
|-------------------|---------------------------|-------|--------|-----------------------|---------------------------|-------|-----------------------|
| d | — g kg ⁻¹ DM — | | | g kg ⁻¹ CP | — g kg ⁻¹ DM — | | g kg ⁻¹ CP |
| 0 | 231 | 197 | 34.0 | 147 | 65.7 | 165 | 716 |
| 5 | 204 | 175 | 29.7 | 145 | 63.1 | 141 | 691 |
| 10 | 204 | 178 | 26.1 | 128 | 59.0 | 145 | 711 |
| 15 | 202 | 180 | 21.5 | 106 | 53.8 | 148 | 732 |
| 20 | 187 | 167 | 20.9 | 113 | 57.5 | 130 | 694 |
| SEM‡ | 4.1 | 4.9 | 1.83 | 10.4 | 1.91 | 4.3 | 10.0 |
| Contrasts§ | <i>P</i> > <i>F</i> | | | | | | |
| Linear | <0.001 | 0.004 | <0.001 | 0.007 | 0.001 | 0.001 | NS¶ |
| Quadratic | NS | NS | NS | NS | NS | NS | NS |
| Cubic | 0.011 | 0.019 | NS | NS | NS | 0.003 | 0.007 |
| Quartic | NS | NS | NS | NS | NS | NS | NS |

†Abbreviations: CP, crude protein; NDSCP, neutral-detergent soluble CP; NDICP, neutral-detergent insoluble CP; RUP, rumen undegradable protein; RDP, rumen degradable protein; DM, dry matter.

‡Standard error of the mean.

§Linear, quadratic, cubic, and quartic effects of days within harvest period.

¶Not significant, *P* > 0.05.

response variables, concentrations declined by 28, 27, and 30%, respectively, between Days 0 and 20. Concentrations of NDICP (g kg⁻¹ DM), NDICP (g kg⁻¹ CP), and RUP all changed in complex curvilinear (*P* ≤ 0.018) patterns with time; however, the magnitude of these changes was again relatively small, and it is unclear how to associate the somewhat erratic nature of these responses with any physiological aspect of plant development or age within harvest period. Expressed on a CP basis, RDP declined linearly (*P*

Table 8. Concentrations of protein components for alfalfa forages harvested from a late-summer harvest period at 0, 5, 10, 15, and 20 d after reaching Stage 2 (Kalu and Fick, 1981) during 2005 at Prairie du Sac, WI.†

| Days from Stage 2 | CP | NDSCP | NDICP | NDICP | RUP | RDP | RDP |
|-------------------|---------------------------|--------|-------|-----------------------|---------------------------|--------|-----------------------|
| d | — g kg ⁻¹ DM — | | | g kg ⁻¹ CP | — g kg ⁻¹ DM — | | g kg ⁻¹ CP |
| 0 | 246 | 209 | 37.1 | 153 | 66.2 | 180 | 731 |
| 5 | 211 | 189 | 21.4 | 101 | 55.1 | 156 | 741 |
| 10 | 217 | 186 | 30.9 | 143 | 63.2 | 154 | 709 |
| 15 | 187 | 154 | 32.7 | 174 | 53.2 | 134 | 715 |
| 20 | 178 | 152 | 25.7 | 144 | 51.9 | 126 | 709 |
| SEM‡ | 5.8 | 6.9 | 3.51 | 18.0 | 2.69 | 4.6 | 9.6 |
| Contrasts§ | <i>P</i> > <i>F</i> | | | | | | |
| Linear | <0.001 | <0.001 | NS¶ | NS | 0.004 | <0.001 | 0.042 |
| Quadratic | NS | NS | NS | NS | NS | NS | NS |
| Cubic | NS | NS | 0.010 | 0.018 | NS | NS | NS |
| Quartic | 0.015 | NS | NS | NS | 0.015 | NS | NS |

†Abbreviations: CP, crude protein; NDSCP, neutral-detergent soluble CP; NDICP, neutral-detergent insoluble CP; RUP, rumen undegradable protein; RDP, rumen degradable protein; DM, dry matter.

‡Standard error of the mean.

§Linear, quadratic, cubic, and quartic effects of days within harvest period.

¶Not significant, *P* > 0.05.

= 0.042) with time, but the magnitude of change between Days 0 and 20 was only 22 g kg⁻¹ CP.

Fall Harvest Period

Responses for specific protein fractions harvested during the FA harvest period followed patterns that were generally consistent with other harvest periods. Concentrations of CP and NDSCP declined by 14 and 17%, respectively, between Days 0 and 20 (Table 9); in both cases, effects were linear (*P* ≤ 0.002) with time. Changes in RDP (g kg⁻¹ DM) were relatively static through Day 10, but declined thereafter by 32 g kg⁻¹ DM, yielding both quadratic (*P* = 0.035) and linear (*P* < 0.001) effects of time. Expressed on a CP basis, RDP (g kg⁻¹ CP) declined from 703 to 635 (g kg⁻¹ CP) between Days 0 and 20, thereby exhibiting the same quadratic (*P* = 0.018) and linear (*P* < 0.001) effects of time observed for RDP (g kg⁻¹ DM). Concentrations of NDICP (g kg⁻¹ DM), NDICP (g kg⁻¹ CP), and RUP exhibited complex relationships with time that were either cubic (RUP; *P* = 0.024), quartic (NDICP, g kg⁻¹ CP; *P* = 0.050), or both (NDICP, g kg⁻¹ DM; *P* ≤ 0.040); however, these changes were generally limited in magnitude, and their practical relevance is questionable.

DISCUSSION

Harvest Period Effects

It is difficult to offer conclusive assessments about the effects of specific harvest periods on partitioning of CP within forage plants, and subsequently, on estimates of ruminal degradability. Generally, each harvest period was somewhat unique; there was a significant (*P* ≤ 0.004) harvest period main effect for five response variables in 2004, and for six response variables (*P* ≤ 0.035) in 2005 (Table 3). However, it is difficult to find any pattern across harvest periods that could be related specifically to expected seasonal climatic trends, such as cooler temperatures in the spring or fall. For instance, concentrations of CP varied widely across harvest periods in 2004 (overall range = 193 to 230 g kg⁻¹ DM; Table 5). Ignoring the harvest period × days interaction, main effect means during 2005 ranged tightly across harvest periods (206 to 210 g kg⁻¹ DM; data not shown), resulting in a nonsignificant (*P* > 0.05) main effect. It seems quite likely that the unique nature of each harvest period may have been influenced heavily by the specific environmental and soil moisture conditions present within that individual harvest period. Despite this somewhat

unique nature of individual harvest periods, there were still strong overall correlations (Table 10) of GDD with NDF ($r = 0.495$, $P = 0.001$), NDICP (g kg^{-1} CP; $r = 0.434$, $P = 0.005$), CP ($r = -0.542$, $P < 0.001$), NDSCP (-0.589 , $P < 0.001$), RDP (g kg^{-1} DM; $r = -0.567$, $P < 0.001$), and RDP (g kg^{-1} CP; $r = -0.425$, $P = 0.006$). Previously, smaller concentrations of RUP have been reported for alfalfa grown under unusually cool conditions (Cassida et al., 2000), but conclusive identification of specific relationships between partitioning of CP and environment may require the use of growth chambers, or other means of artificial climate control.

Day Effects

Neutral-Detergent Insoluble Crude Protein

Compared to harvest-period effects, the effects of plant age within each harvest period, defined as days from Stage 2 (Kalu and Fick, 1981), were much more consistent. During 2004, CP declined with time, exhibiting both quadratic and linear ($P < 0.001$) effects (Table 5). Declining concentrations of CP have been observed previously within maturing alfalfa forages (Broderick et al., 1992; Hoffman et al., 1993; Cassida et al., 2000). Our results suggest that this response is largely a function of declining CP within the cell solubles, and is relatively independent of cell wall-associated protein (NDICP). This observation is supported by the strong positive correlation ($r = 0.979$; $P < 0.001$) between CP and NDSCP, and the absence of correlation ($r = 0.118$, $P = 0.467$) between NDSCP and NDICP (Table 10).

During 2004, NDICP was very consistent (range = 26.3 to 29.0 g kg^{-1} DM), exhibiting no polynomial relationship with days within harvest period ($P > 0.05$). These concentrations for NDICP are comparable to estimates for other alfalfa forages (Coblentz et al., 1998, 1999). Furthermore, concentrations of NDICP (g kg^{-1} DM) for alfalfa forages may not be affected by leaf to stem ratios; Coblentz et al. (1998) found virtually identical concentrations of NDICP within leaf, stem, and whole-plant tissue of alfalfa harvested at 10% bloom, which may partially explain the relative stability of this fraction as alfalfa plants aged within harvest period. Elevated ambient temperatures are widely known to accelerate plant maturation and decrease leaf to stem ratios (Buxton and Fales, 1994), and accumulated GDD ranged widely within the eight harvest periods during the study (range = 264 to 799 GDD; Table 1). However, throughout both 2004 and 2005, NDICP (g kg^{-1} DM) was not correlated with GDD ($r = 0.089$, $P = 0.672$; Table 10), further suggesting that concentrations of this fraction are relatively independent of plant maturity.

It is important to note two other related points. First, the concentration of NDICP (g kg^{-1} DM) within any for-

Table 9. Concentrations of protein components for alfalfa forages harvested from a fall harvest period at 0, 5, 10, 15, and 20 d after reaching Stage 2 (Kalu and Fick, 1981) during 2005 at Prairie du Sac, WI.[†]

| Days from Stage 2 | CP | NDSCP | NDICP | NDICP | RUP | RDP | RDP |
|------------------------|-----------------------|-------|-----------------|-----------------------|-------|-----------------------|--------|
| d | g kg ⁻¹ DM | | | g kg ⁻¹ CP | | g kg ⁻¹ CP | |
| 0 | 220 | 188 | 32.1 | 146 | 65.4 | 155 | 703 |
| 5 | 223 | 181 | 41.5 | 187 | 67.6 | 155 | 696 |
| 10 | 218 | 187 | 30.4 | 141 | 65.5 | 152 | 699 |
| 15 | 193 | 160 | 32.2 | 168 | 61.6 | 131 | 679 |
| 20 | 189 | 156 | 33.2 | 176 | 69.0 | 120 | 635 |
| SEM [‡] | 5.0 | 6.7 | 2.45 | 13.8 | 1.91 | 4.6 | 9.6 |
| Contrasts [§] | | | | $P > F$ | | | |
| Linear | <0.001 | 0.002 | NS [¶] | NS | NS | <0.001 | <0.001 |
| Quadratic | NS | NS | NS | NS | NS | 0.035 | 0.018 |
| Cubic | NS | NS | 0.026 | NS | 0.024 | NS | NS |
| Quartic | NS | NS | 0.040 | 0.050 | NS | NS | NS |

[†]CP, crude protein; DM, dry matter; NDSCP, neutral-detergent soluble CP; NDICP, neutral-detergent insoluble CP; RUP, rumen undegradable protein; RDP, rumen degradable protein.

[‡]Standard error of the mean.

[§]Linear, quadratic, cubic, and quartic effects of days within harvest period.

[¶]Not significant, $P > 0.05$.

age is actually the product of two dynamic factors: (i) concentrations of protein within the insoluble NDF residue; and (ii) concentrations of NDF within the forage. Within this study, concentrations of NDF were not correlated ($r = -0.224$, $P = 0.165$; Table 10) with NDICP (g kg^{-1} DM). However, NDF increased over time within harvest periods, exhibiting inconsistent polynomial effects across harvest periods during 2004, and a linear ($P < 0.001$) relationship for all harvest periods combined during 2005 (Table 4). Therefore, when whole-plant concentrations of NDICP (g kg^{-1} DM) remain stable as alfalfa plants age within each harvest period, concentrations of CP within the isolated insoluble NDF residues must be fluid over same time interval.

Second, NDICP is frequently reported as a percentage or proportion of CP, rather than DM. During 2004, our estimates of NDICP (g kg^{-1} CP) increased linearly ($P = 0.001$) by 21% between Days 0 and 20 (Table 5). However, this linear increase is primarily an artifact of decreasing concentrations of CP within the whole-plant forage, rather than substantial changes in the NDICP (g kg^{-1} DM) pool. This premise is supported by an overall negative correlation ($r = -0.347$, $P = 0.028$; Table 10) between CP and NDICP (g kg^{-1} CP).

Rumen Undegradable Protein

During 2004, concentrations of RUP exhibited a quadratic ($P = 0.002$; Table 5) relationship with days within harvest period; however, in practical terms, this response was quite similar to that exhibited by NDICP (g kg^{-1} DM), and suggests this fraction, expressed on a g kg^{-1} DM basis, is relatively independent of plant maturity. During

2004, concentrations of RUP ranged narrowly over time from 60.4 to 66.5 g kg⁻¹ DM, and estimates for Days 0 and 20 differed by only 1.2 g kg⁻¹ DM. Furthermore, RUP was not correlated with either GDD ($r = 0.003$, $P = 0.985$) or NDF ($r = -0.075$, $P = 0.647$), both of which would be expected to have close associations with plant maturity.

Relatively stable estimates of RUP over days within harvest period are consistent with responses for NDICP (g kg⁻¹ DM), and are not necessarily surprising. Protein that is insoluble in neutral detergent, but soluble in acid detergent, degrades slowly in the rumen because of its presumed association with the cell wall, and it is a major contributor to the pool of available protein that escapes the rumen intact (Sniffen et al., 1992). Furthermore, relatively small changes in RUP pools for maturing plants have been reported previously. Mitchell et al. (1997) reported that RUP concentrations for smooth brome grass (*Bromus inermis* Leyess.) and intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey], both of which possess the C₃ pathway of carbon fixation, were largely unaffected by morphological development. When converted to a gram per kilogram of DM basis, estimates of RUP made by Hoffman et al. (1993) for smooth brome grass and orchardgrass (*Dactylis glomerata* L.) changed little across the second node, boot, and fully headed stages of growth; respective concentrations at these growth stages were 47, 46, and 43 g kg⁻¹ DM for smooth brome grass, and 39, 40, and 40 g kg⁻¹ DM for orchardgrass. For alfalfa,

Cassida et al. (2000) suggested that delaying harvest to increase plant maturity resulted only in small gains in RUP, and these gains came at the expense of other measures of forage quality, thereby rendering this approach counterproductive. Similarly, Hoffman et al. (1993) evaluated alfalfa for RUP at the late-vegetative, late-bud, and midbloom stages of growth; after converting to a g kg⁻¹ DM basis, respective estimates for these forages were 43, 46, and 49 g kg⁻¹ DM, thereby indicating only minor change with increasing maturity.

It should again be noted that many researchers and nutritionists prefer to express RDP or RUP as a percentage or proportion of CP (National Research Council, 1996, 2001). In some cases this complicates interpretation. If our estimates for RUP (g kg⁻¹ DM) were converted to a CP basis, RUP (g kg⁻¹ CP) would increase with plant maturity, which has been noted by many other researchers working with both grasses and legumes (Mullahey et al., 1992; Hoffman et al., 1993; Mitchell et al., 1997; Cassida et al., 2000). However, this response again is primarily an artifact of declining concentrations of whole-plant CP, rather than changes in the actual RUP pool (g kg⁻¹ DM) itself.

Rumen Degradable Protein

During 2004, RDP (g kg⁻¹ DM) decreased by 26% in quadratic ($P = 0.026$) and linear ($P < 0.001$) relationships with days within harvest period (Table 4). This

Table 10. Pearson correlation coefficients for interaction means (harvest period × days within harvest period; $n = 40$) relating growing degree days (GDD), neutral-detergent fiber (NDF), crude protein (CP), neutral-detergent soluble CP (NDSCP), neutral-detergent insoluble CP (NDICP), rumen undegradable protein (RUP), and rumen degradable protein (RDP) for alfalfa forages harvested during 2004 and 2004.

| Index | Statistic [†] | GDD [‡] | g kg ⁻¹ DM [§] | | | | g kg ⁻¹ CP | | g kg ⁻¹ DM | | g kg ⁻¹ CP |
|------------------------------|------------------------|------------------|------------------------------------|--------|--------|--------|-----------------------|--------|-----------------------|--------|-----------------------|
| | | | NDF | CP | NDSCP | NDICP | NDICP | RUP | RDP | RDP | |
| GDD | r | – | 0.495 | –0.542 | –0.589 | 0.069 | 0.434 | 0.003 | –0.567 | –0.425 | |
| | P | – | 0.001 | <0.001 | <0.001 | 0.672 | 0.005 | 0.985 | <0.001 | 0.006 | |
| NDF, g kg ⁻¹ DM | r | | – | –0.831 | –0.821 | –0.224 | 0.344 | –0.075 | –0.840 | –0.592 | |
| | P | | – | <0.001 | <0.001 | 0.165 | 0.030 | 0.647 | <0.001 | <0.001 | |
| CP, g kg ⁻¹ DM | r | | | – | 0.979 | 0.319 | –0.347 | 0.276 | 0.947 | 0.529 | |
| | P | | | – | <0.001 | 0.045 | 0.028 | 0.084 | <0.001 | 0.001 | |
| NDSCP, g kg ⁻¹ DM | r | | | | – | 0.118 | –0.530 | 0.173 | 0.960 | 0.603 | |
| | P | | | | – | 0.467 | <0.001 | 0.287 | <0.001 | <0.001 | |
| NDICP, g kg ⁻¹ DM | r | | | | | – | 0.774 | 0.550 | 0.147 | –0.239 | |
| | P | | | | | – | <0.001 | <0.001 | 0.364 | 0.138 | |
| NDICP, g kg ⁻¹ CP | r | | | | | | – | 0.351 | –0.478 | –0.582 | |
| | P | | | | | | – | 0.027 | 0.002 | <0.001 | |
| RUP, g kg ⁻¹ DM | r | | | | | | | – | –0.046 | –0.664 | |
| | P | | | | | | | – | 0.777 | <0.001 | |
| RDP, g kg ⁻¹ DM | r | | | | | | | | – | 0.770 | |
| | P | | | | | | | | – | <0.001 | |

[†]Correlation statistics: r , correlation coefficient; P , probability of a greater $|r|$.

[‡]Growing degree days were calculated daily by subtracting 5°C from the average of the maximum and minimum temperatures for that day, and then summing over days within each harvest period.

[§]DM, dry matter.

response over time occurred in parallel with declining concentrations of whole-plant CP, but is most likely associated specifically with a shrinking pool of NDSCP (g kg^{-1} DM). Over the entire study, both CP and NDSCP exhibited very strong positive correlations ($r \leq 0.947$, $P < 0.001$; Table 10) with RDP (g kg^{-1} DM), thereby establishing further the parallel relationship existing between these three fractions. Sniffen et al. (1992) divided the NDSCP pool into three subfractions: (i) nonprotein N; (ii) proteins soluble in borate-phosphate buffer (Krishnamoorthy et al., 1983); and (iii) proteins insoluble in borate-phosphate buffer, but soluble in neutral detergent. Of these, the first two fractions are degraded and/or converted to ammonia in the rumen. The final fraction is incompletely degraded in the rumen, and its fate is dependent on relative rates of degradation and passage. Given the nature of these subfractions, and the known rapid rates of ruminal degradation for alfalfa proteins (0.18 to 0.23 h^{-1} , Hoffman et al., 1993; 0.21 h^{-1} , Coblenz et al., 1998), the positive relationship between NDSCP (g kg^{-1} DM) and estimates of RDP (g kg^{-1} DM) is expected.

When expressed as a proportion of CP, RDP (g kg^{-1} CP) for 2004 (Table 5) declined linearly ($P < 0.001$) from 720 to 659 g kg^{-1} CP during the 20-d sampling period. Expressing RDP on this basis mediates the response, and can complicate interpretation, because both RDP and total CP pools (g kg^{-1} DM) decline simultaneously with days within harvest period. The declining pattern over time is consistent with other work; however, estimates determined by in situ methodology (Hoffman et al., 1993) yielded slightly greater values for alfalfa than those in our study (839, 774, and 721 g kg^{-1} CP at late-vegetative, late-bud, and midbloom stages of growth, respectively). In situ and enzymatic analytical approaches both have limitations (Broderick, 1994), and they are known to give results that vary slightly. In a previous study, a 48-h incubation with *S. griseus* protease underestimated RDP in high-quality legumes relative to estimates obtained from in situ techniques (Coblenz et al., 1999). Based on the linear relationship between *S. griseus* protease and in situ estimates of RDP identified in that work, a hypothetical forage with a RDP concentration of 800 g kg^{-1} CP obtained by in situ techniques would likely exhibit a concentration of about 712 g kg^{-1} CP by the *S. griseus* protease procedure, which is an underestimation of approximately 11%. Given that we observed RDP estimates as low as 635 g kg^{-1} CP (Table 9) for alfalfa forages in this trial, it is likely that some similar underestimation (relative to in situ estimates) occurred in this study.

It should be noted that the discrepancy between enzymatic and in situ determinations of RDP is partially procedural. In situ estimates of RDP require inputs of both

ruminal degradation and particulate passage rates (Ørskov and McDonald, 1979). In contrast, enzymatic estimates are independent of passage rate. In the work discussed previously (Coblenz et al., 1999), 20 diverse forages were evaluated in situ within steers consuming a basal diet of smooth brome-grass hay; the mean particulate passage rate of that diet was 0.03 h^{-1} . Had the basal diet been more reflective of those consumed by lactating dairy cows, a more rapid passage rate (0.06 h^{-1} ; Broderick et al., 1992; Hoffman et al., 1993) would be likely, thereby resulting in increased (in situ) ruminal escape, and better agreement between methods.

Other Considerations

The discussion of day within harvest period effects is complicated by the interaction ($P \leq 0.020$) of main effects that was observed for all protein-related response variables during 2005 (Table 3). For 2004, there were no interactions ($P > 0.05$) of main effects, and responses over time for all harvest periods combined were either linear, quadratic, or both ($P \leq 0.026$). No higher-ordered polynomial effects were observed for any protein-related response variable. In contrast, numerous cubic ($P \leq 0.030$) and/or quartic ($P \leq 0.050$) effects were observed within individual harvest periods during 2005 (Tables 6–9). These complex responses over time are difficult to interpret, and attempts to do so would be speculative; however, close inspection of general trends over time within individual harvests suggest that the results for 2005 could best be described as somewhat erratic, rather than truly divergent from 2004. Most of the general trends over time described for 2004 can be observed within individual harvests for 2005; these include declining concentrations of CP, NDSCP (g kg^{-1} DM), RDP (g kg^{-1} DM or CP), and relatively consistent pools of NDICP (g kg^{-1} DM) and RUP. It remains unclear whether the more erratic responses observed during 2005 occurred in response to the specific weather patterns during each growing cycle, which included extended periods of precipitation deficit, or for other reasons.

CONCLUSIONS

Generally, aging within harvest period reduced concentrations of rumen degradable protein within alfalfa forages, but the effects of SP, ES, LS, and FA harvest periods were somewhat erratic, and likely were influenced heavily by climatic and/or soil moisture conditions within that specific growth cycle. Rumen degradable protein declined over time within harvest period when it was expressed as a proportion of both whole-plant DM and CP. In contrast, concentrations of CP insoluble in neutral detergent, and presumably associated with the cell wall, remained relatively stable across harvest periods, as well as across days within each harvest period. Because this CP fraction is generally resistant to ruminal degradation, and comprises a substantial proportion of the

total undegradable protein pool, concentrations of rumen undegradable protein, expressed as a proportion of whole-plant DM, also remained relatively stable across all treatment factors. Given the relatively stable concentrations of proteins associated with the cell wall, declines in concentrations of rumen degradable protein were most likely related to concomitant reductions of highly-degradable, cell-soluble protein that also were observed as a function of the aging within each harvest period.

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