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Estimation of Rumen Undegradable Protein in Forages by Using Neutral Detergent Insoluble Nitrogen at a Single In Situ Incubation Time Point

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In situ incubation of forages for a single time point, equivalent to 75% of the mean retention time, accurately estimated UIP using NDIN

Summary

Neutral detergent insoluble nitrogen (NDIN) was used as a direct estimate of UIP. Forage samples collected from upland range and subirrigated meadow sites over the summer were incubated in situ for a time equivalent to a mean retention time estimated from the digestibility of the forage plus 10 hour to account for a lag in passage of particles from the rumen. Samples also were incubated for 75% of the estimated total mean retention time. The UIP values obtained from the fractional rates of degradation and passage were highly correlated with those estimated from samples incubated for 75% of total mean retention time while incubating the samples longer tended to underestimate the UIP fraction.

Introduction

Previous research at the University of Nebraska demonstrated that neutral detergent insoluble nitrogen (NDIN) can be used as a direct estimate of UIP in forages (*1997 Beef Cattle Report,* pp.38-39). The standard method uses a first order disappearance model to estimate the potentially digestible fraction that escapes rumen degradation.

The first order disappearance model assumes ingested particles are capable of passing immediately out of the rumen. This may not be the case of particles that are too large or buoyant to reach the reticulo-omasal orifice and escape the rumen. The consequence of not accounting for a lag in passage, time during which particles may be digested but cannot escape, is that UIP may be overestimated. It has been suggested that this lag in passage is relatively constant, and it is approximately 10 hour.

Using a data set from the University of Nebraska, we compared the results obtained by using the fractional rates of passage and digestion and accounting for passage lag time of 10 hours with those obtained from a single incubation time equivalent to total mean retention time (TMRT). The UIP values were lower when a single time point was used than when using fractional rates of passage and degradation. We also observed that values obtained by the two methods approached similarity if a TMRT analogous to 75% of TMRT was used. The objective of this study was to compare UIP results and rates of NDIN degradation obtained from forage samples incubated for the estimated TMRT and for a time equivalent to 75% of TMRT.

Procedure

Forage Samples

Two types of forage were evaluated in this study: upland native range (Range) and subirrigated meadow (Meadow). Forages were grown at the Gudmunsen Sandhills Laboratory (GSL) of the University of Nebraska, near Whitman, Neb. The dominant grass species in Range were: little bluestem, prairie sandreed, sand bluestem, switchgrass, sand lovegrass, indiangrass, and grasslike plants. Dominant species in Meadow were: Kentucky bluegrass, slender wheatgrass, smooth bromegrass, timothy, reed canarygrass, redtop, several species of sedges and clover. Samples were collected from two pastures on each site (Meadow and Range) with three esophageally fistulated cows. Collections were carried out on May 25, June 22, July 20, Aug. 17 and Sept. 21. Esophageal masticate samples were frozen immediately, later freeze-dried and ground to pass through a 2-mm screen, and a subsample was ground through a 1-mm screen for further determination of in vitro digestibility. Samples from the same pasture and period were composited on a DM basis.

In Situ Procedures

Two ruminally cannulated steers were housed in individual pens and offered a total mixed ration of 70% brome hay and 30% concentrate. Rumen degradability of protein in the experimental forages was determined by incubating duplicate 5 x 10 cm dacron bags filled with 1.25 g of forage in the rumen of each steer. Experimental incubation times were determined from IVDMD. First, using the following equation rate of passage (Kp) was estimated:

$$kp\ (\%/h) = 0.07\ IVDMD\ (\%) - 0.20.$$

Then mean retention time was calculated as the inverse of kp. A 10-hour lag time was added to the estimated mean retention time and designated total mean retention time (TMRT), the total time particles would be subjected to

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degradation. Samples were incubated for 10 hours, the calculated TMRT, a period equivalent to 75% of the TMRT, and 96 hours. The estimated mean retention time for forages collected in May and Meadow in June was about 31 hours. For Range samples collected in June and all samples collected in July and August, the estimated mean retention time was 35 hours and 40 hours for those Range samples collected in September.

After incubation, sample residues were refluxed with neutral detergent solution in an Ankon Fiber Analyzer and analyzed for N content by the combustion method using a nitrogen analyzer.

Calculations

The NDIN content was calculated for the original forage sample and for each in situ forage residue, thus allowing the establishment of a degradation curve for NDIN. The UIP as a % DM of the original sample was calculated as NDIN (% of DM) in the residue of samples incubated for the estimated TMRT multiplied by 6.25 to convert N to crude protein equivalents.

The original NDIN pool was measured on 0-hour samples. The portion of NDIN remaining in bags incubated for 96 hours was considered to be the ruminally unavailable fraction. Potentially degradable NDIN was determined as total NDIN – NDIN content of the unavailable portion. Rates of ruminal degradation (k_d) for each in situ CP fraction were calculated by using a first order disappearance model. The k_d was calculated as the slope of the regression of the natural logarithm of NDIN remaining (after NDIN content of the unavailable fraction was subtracted) against time.

Data were analyzed using the MIXED procedure of SAS. Type of forage (Meadow and Range), collection period (May through September) and incubation time (10 hour, 0.75 TMRT and TMRT) were included in the model as fixed effects, and pasture, nested within forage type, as random effect. Average rates of protein degradation were calculated from the two duplicate samples and the two steers.

Table 1. Original CP content and undegraded protein as a percentage of DM of range and meadow samples collected from May to September.

Item	Original CP[a]	Incubation time (hour) 0[b]	10[b]	.75 TMRT[b]	TMRT[b]	96
Range						
May	12.0	5.27	3.90	2.02	1.83	0.95
June	9.7	3.69	2.94	1.71	1.21	1.40
July	9.5	3.15	2.56	1.35	1.08	0.98
August	9.3	3.08	2.10	0.91	0.91	1.62
September	9.4	2.18	1.67	0.76	0.48	2.18
Meadow						
May	13.7	7.81	5.11	1.79	1.77	0.74
June	12.2	5.55	4.03	1.98	2.27	0.93
July	12.8	5.40	2.83	0.99	1.08	1.39
August	12.4	3.87	2.30	1.00	0.90	1.47
September	8.4	2.54	1.63	0.76	0.28	1.33

[a]Percentage of DM.
[b]Undegraded protein as a percentage of DM corrected for 96 hour values.

Table 2. Rates of degradation (%/hour) of protein of summer range and meadow incubated from 0 to 10 hour, 10 to a time equivalent to 75% of TMRT and 75% of TMRT to TMRT.

Item	0-10[a]	10-.75 TMRT[b]	.75 TMRT-TMRT[c]
Range			
May	3.03	5.15	1.18
June	2.23	3.24	4.19
July	1.86	3.74	2.68
August	3.93	4.86	0.18
September	2.69	3.75	9.02
Meadow			
May	4.33	8.38	0.19
June	3.18	5.41	-1.60
July	6.36	5.66	0.57
August	5.21	4.91	1.73
September	4.27	3.64	11.06

[a]Rate of protein degradation from 0 to 10 hours inubation.
[b]Rate of protein degradation from 10 to .75 TMRT.
[c]Rate of protein degradation from .75 TMRT to TMRT.

Results

The protein undegraded at the various times is shown in Table 1. Protein degradability was greater for Meadow than Range ($P < 0.05$). Protein degradability decreased from May to September.

Table 2 shows rates of protein degradation for the first 10 hours of incubation (lag time) and the period following the lag time through a time point equivalent to 75% of the estimated TMRT. Two significant interactions: month x incubation time ($P < 0.05$) and month x forage type ($P = 0.09$) were observed. The protein of forages collected in May and June was degraded more slowly from 0 to 10 hours than from 10 hours to 0.75 TMRT ($P < 0.05$), but rates of degradation were not significantly different for the rest of the collection periods ($P > 0.1$). This trend for protein of samples collected early in the season to be more resistant to initial degradation may be indicative of some lag in NDIN digestion. Since NDIN is the protein fraction associated with the cell wall, a lag in fiber digestion when the microbes attach to the fiber but no digestion occurs, might affect the initial availability of protein.

Regardless of the length of the incubation, meadow protein degraded more rapidly than range early in the season (May to July; $P < 0.1$). This tendency for meadow to degrade more rapidly than range during this phase of incubation may be indicative of the differences across these types of grass that affect the availability of protein to microbial degradation. Warm-season grasses

Table 3. UIP content (% DM) of summer upland range and meadow estimated by three different approaches.

Item	Equation[a]	.75 TMRT[b]	TMRT[b]
Range			
May	2.87	3.02	2.76
June	3.07	3.11	2.59
July	2.35	2.33	2.08
August	2.52	2.54	2.30
September	2.97	2.94	2.45
Meadow			
May	2.63	2.53	2.53
June	2.74	2.90	3.18
July	2.54	2.48	2.47
August	2.46	2.47	2.39
September	2.11	2.08	1.61

[a]UIP = 0 hour value (Table 1) * (kp/kp + kd) + 96 hour value (Table 1), corrected for passage lag time.
[b]In situ incubation for 75 % of TMRT or TMRT.

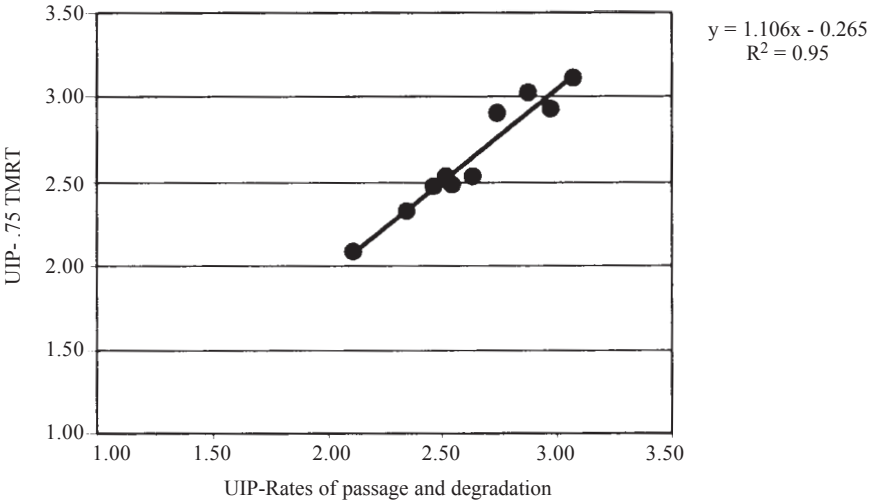

Figure 1. Relationship between forage UIP content calculated either by a single in situ incubation time point equivalent to 75% of the total ruminal mean retention time or by the fractional rates of digestion and passage.

dominating the range site contain a rigid, thick-walled parenchyma bundle sheath that degrades more slowly than the fiber in cool-season species (meadow) which may protect protein from microbial degradation. In addition, lignification of the parenchyma bundle sheath with increasing maturity would make protein within this structure potentially less digestible.

When comparing rates of protein degradation for 10 hours to 0.75 TMRT and 0.75 TMRT-TMRT, rates were significantly slower for the last part of the incubation for forages collected from May through August ($P < 0.05$); however, a dramatic increase in the 0.75 TMRT-TMRT rate of degradation was observed for the September forages (10 versus 1.15%/hour for September and May through August average respectively for average of Range and Meadow). When comparing the values for the two last points of the degradation curve, 0.75 TMRT and TMRT, values for the two times did not differ from May through August ($P > 0.1$), but the potentially degradable fraction remaining was significantly higher at 0.75 TMRT than at TMRT in September ($P < 0.001$); Table 1). The similar contents of NDIN in the residues when forages were incubated for 0.75 TMRT and TMRT as well as lower rates of digestion of NDIN during 0.75 TMRT-TMRT than 10-0.75 TMRT indicate that most of the potentially digestible NDIN was already degraded at the 0.75 TMRT point. September forages were the exception. Degradation appeared to continue from 0.75 TMRT to TMRT. This could have been due to forages becoming dormant and, consequently, more resistant to digestion. However, in our experiment the potentially degradable NDIN fraction at 0.75 TMRT was already very small for the September forages.

Undegraded intake values obtained from the competition of the fractional rates of digestion and passage from the rumen as used in many models may be a more accurate estimate than a single time point incubation. Therefore, values obtained from such a model of the competition of kp and kd with the addition of a passage lag were regressed linearly on corresponding estimates obtained from a single incubation time point, either TMRT or 0.75 TMRT.

The UIP values obtained from the three approaches are shown in Table 3. UIP estimates from samples incubated for 0.75 TMRT were highly correlated with those calculated from fractional rates of digestion and passage ($R^2 = .95$; Figure 1). The slope of the regression line was 1.1064 (SE = 0.09), and was not different from 1 ($P < 0.05$). The intercept was equal to –0.2652, and it was not different from 0 ($P = 0.32$); therefore, assuming an intercept equal to 0, the slope is 1.0066 and the R^2 of the regression is 0.999. This clearly indicates that the two methods yielded similar estimates of UIP. On the contrary, incubating samples longer (TMRT) tended to underestimate UIP ($R^2 = 0.53$).

The overall results of this trial suggest using a single incubation time point equivalent to 75% of the TMRT estimated from IVDMD and accounting for a passage lag time would give accurate UIP estimates as well as rates of NDIN degradation.

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