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Summary

Digestibility of undegradable intake protein of smooth bromegrass, birdsfoot trefoil, and heat-treated alfalfa was determined using the mobile nylon bag technique. Undegradable intake protein (UIP) was determined using neutral detergent insoluble protein at 75% of the total mean retention time; 1.82 and 1.71 in June and July for brome and 1.30 and 1.94 in June and July for birdsfoot trefoil. Digestibility (%) of the UIP in brome was 38.6 and 27.1 in June and July and in birdsfoot trefoil 21.1 and 25.1. The UIP (% DM) of alfalfa dried to simulate dehydrated, sun-cured, and fresh alfalfa, was 3.13, 2.10, and 1.84. Digestibility (%) of UIP was highest for dehydrated (46.4) followed by sun-cured (25.6) and fresh alfalfa (14.7). The undegradable intake protein content and digestibility of the UIP of forages is low.

Introduction

Protein evaluation systems such as the NRC model for beef (1996) and dairy (2001) cattle recognize intestinal digestibilities of proteins may differ by source. Prior to the 2001 revision of the dairy NRC, a constant digestibility of 80% was used for the undegradable intake protein (UIP) of all feedstuffs. The 1996 beef NRC still uses a constant digestibility of 80% because of a lack of information available on UIP digestibility; however, the dairy NRC (2001) now uses variable digestibilities from 50% to 100%.

The length of incubation of forages in the rumen can significantly influence the measured intestinal digestibility of UIP because protein flowing from the rumen is greater at shorter incubation times and indigestible protein is the same. Many values reported in the literature for UIP and the digestibility of UIP in forages are based on ruminal incubations of 16 hours or less, which may not reflect true residence time of forage particles in the rumen. Digestibilities of UIP in forages might be overestimated when rumen incubations are too short. The objectives of the trial were: 1) to evaluate the protein characteristics of birdsfoot trefoil (BFT) and smooth bromegrass and 2) to determine the effect of heat treatment on the UIP content and digestibility in alfalfa.

Procedure

In the first experiment, birdsfoot trefoil and smooth bromegrass clip samples were collected from two fields on two dates (June and July, 2003) from a smooth bromegrass pasture interseeded with BFT at the University of Nebraska Agricultural Research and Development Center near Mead, Nebraska. Samples were frozen at -4ºC, freeze dried (–50ºC) for 72 hours, and ground through a 2 mm screen for in situ incubation and a 1 mm screen for laboratory analysis.

In vitro dry matter disappearance (IVDMD) was determined on samples and used to estimate the rate of passage (kp) of each of the forages using the following equation: $kp = 0.07 * IVDMD\ (\%) - 0.20$. The kp was then used to determine the mean retention time (MRT = 1/kp). A 10-hour passage lag was added to the MRT to yield the total mean retention time (TMRT).

Two ruminally cannulated heifers (1226 lb) were used to incubate 5 × 10 cm dacron bags with 50 µm pore size. Bags containing 1.25 g of air-dry forage ground through a 2 mm screen were heat-sealed. A mixed ration of 70% bromegrass hay and 30% concentrate was fed twice daily for a total intake of 1.5% BW. Duplicate bags were incubated at each time point and replicated over two days. Four 75% TMRT bags per heifer also were incubated on these two occasions for the intestinal incubation.

In vitro dry matter disappearance (%) of BFT in June and July was 74.5 and 64.4, respectively, which produced 75% TMRT incubation time points of 22.5 hours in June and 25.0 hours in July. Smooth bromegrass was incubated for 26.3 hours in June (59.9% IVDMD) and 28.9 hours in July (52.9% IVDMD), based on the calculated 75% TMRT. The 75% TMRT bags were washed in a washing machine for 0.25 hours using five rinse cycles consisting of a 1 minute agitation and a 2 minute spin following incubation in the rumen. Bags were subsequently refluxed in neutral detergent fiber solution to remove microbial contamination and determine the NDIN in the residue.

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Intestinal 75% TMRT bags were not washed but frozen until insertion into the duodenum. Ruminally incubated bags (75% TMRT) set aside for duodenal insertion were pre-incubated in a pepsin and HCl solution at 37ºC for 3 hours to simulate abomasal digestion. In Experiment 1, two duodenally cannulated steers (1305 lb) were used to incubate these bags over eight days. Steers were fed a mixed diet of 70% bromegrass hay and 30% concentrate twice daily at 1.5% of BW. Bags were inserted into the duodenum 2 hours post-feeding at a rate of 1 bag every 0.1 hour for a total of eight bags per steer per day. Bags were collected in the feces beginning 12 hours after insertion and frozen until all bags were collected. Bags were machine washed and refluxed in neutral detergent fiber solution to correct for microbial contamination of the forage residues. Residues were analyzed for N using a combustion method.

In a second experiment, alfalfa samples from plots fertilized with an average of 59 lb N/acre and 178 lb N/acre were used to evaluate effects of heat treatment and N fertilization on protein degradability in the rumen and the resulting digestibility of the UIP of alfalfa in the small intestine. Alfalfa was frozen at -4ºC until drying methods were applied. Drying methods were simulated in the laboratory and included sun-cured, dehydrated, and freeze-dried (fresh) alfalfa. Sun curing was simulated by drying the sample in a forced-air oven at 50ºC for 15 hours. The process of dehydration was simulated by drying the sample in a forced-air oven at 100ºC for 10 hours. Dry samples were ground through a 2 mm screen for in situ analysis and a 1 mm screen for lab analysis.

Two ruminally cannulated steers (1451 lb) fed a mixed ration of 65% alfalfa and 35% dry rolled corn twice daily for a total intake of 2% of BW were used to incubate quadruplicate 75% TMRT bags. Eight 75% TMRT bags were also incubated in the rumen in preparation for intestinal insertion. Dehydrated, sun-cured, and freeze-dried alfalfa averaged 70.0% IVDMD, and the calculated 75% TMRT point was 23.5 hours. Two duodenally cannulated steers (1451 lb) fed a mixed diet of 65% alfalfa hay and 35% dry rolled corn twice daily at 2% of BW were used to incubate bags over eight days. A total of 12 bags/steer were incubated each day at a rate of 1 bag every 0.1 hours. Bags were collected and handled as in Experiment 1.

Data were analyzed as a completely randomized design using the MIXED procedure of SAS with treatments set up in a 2 × 2 factorial arrangement with forage and date as fixed effects in Experiment 1. In Experiment 2, treatments were arranged in a 2 × 3 factorial with N level and heat treatment as fixed effects in the model. Animal, day, and week were treated as random effects in both experiments.

Table 1. Protein characteristics of smooth bromegrass and birdsfoot trefoil in June and July.

	Smooth Bromegrass		Birdsfoot Trefoil		
	June	July	June	July	SEM[a]
CP, % DM	15.9	9.9	24.7	16.1	
UIP, % DM[b]	1.82[f]	1.71[fh]	1.30[g]	1.94[fi]	0.08
TT IDP, % DM[c]	1.11[f]	1.24[g]	1.02[h]	1.45[i]	0.04
Digestibility of UIP, %[d]	38.6[f]	27.1[gj]	21.1[h]	25.1[ij]	1.8
DUIP, % DM[e]	0.70[f]	0.46[gj]	0.28[h]	0.47[ij]	0.04

[a]Standard error of the mean.
[b]Undegradable Intake Protein (UIP, % DM) = [NDIN at 75% total mean retention time (TMRT) * 6.25]/sample DM. Forage × Date $P < 0.01$.
[c]Total Tract Indigestible Dietary Protein (TT IDP, % DM) = (fecal NDIN * 6.25)/sample DM. Forage × Date $P < 0.01$.
[d]Digestibility of UIP = 1 - (TT IDP/UIP). Forage × Date $P < 0.01$.
[e]Intestinal Disappearance of UIP (DUIP, % DM) = UIP - TT IDP. Forage × Date $P < 0.01$.
[f,g,h,i,j]Means within a row with different superscripts differ ($P < 0.05$).

Table 2. Effect of heat treatment on the undegradable intake protein (UIP), total tract indigestible protein (TT IDP), and digestibility of UIP of alfalfa.

	Heat Treatment[a]			
	Dehydrated	Sun-cured	Freeze-dried	SEM[b]
CP, % DM	20.5	21.1	20.9	
UIP, % DM[c]	3.13[f]	2.10[g]	1.84[h]	0.08
TT IDP, % DM[d]	1.66[f]	1.54[g]	1.57[g]	0.04
Digestibility of UIP, %[e]	46.4[f]	25.6[g]	14.7[h]	1.8

[a]Heat Treatment applied: Dehydrated, 100ºC for 10 hours; Sun-cured, 50ºC for 15 hours; Freeze-dried, -50ºC for 72 hours.
[b]Standard error of the mean.
[c]Undegradable Intake Protein (UIP, % DM) = [NDIN at 75% total mean retention time (TMRT) * 6.25]/sample DM.
[d]Total Tract Indigestible Dietary Protein (TT IDP, % DM) = (fecal NDIN * 6.25)/sample DM
[e]Digestibility of UIP = 1 - (TT IDP/UIP).
[f,g,h]Means within a row with unlike superscripts differ $P < 0.05$.

Results

Protein characteristics of smooth bromegrass and BFT are shown in Table 1. There was a significant forage x date interaction ($P < 0.01$) for the UIP, IDP, digestibility of UIP, and DUIP in Experiment 1. Undegradable intake protein (% DM) of smooth bromegrass was similar

($P = 0.11$) in June (1.82) and July (1.71); however, the UIP (% DM) of BFT increased 49% from 1.30 in June to 1.94 in July ($P < 0.01$). Total tract IDP (% DM) of smooth bromegrass increased 12% from 1.11 in June to 1.24 in July ($P < 0.01$); however, the increase in IDP of BFT was greater (42%) as the IDP (% DM) in June and July were 1.02 and 1.45, respectively.

The tannin concentration of BFT in July may have protected a larger fraction of the CP later in the summer as the DUIP (% DM) was 0.47 percentage units in July but only 0.28 percentage units in June ($P < 0.01$). Tannins offer some protection from protein degradation in the rumen as a result of the tanning of proteins and inactivating enzymes. These tannin-protein complexes can potentially be digested in the lower tract. The increase in IDP in July for smooth bromegrass resulted in a reduction in DUIP (% DM) from 0.70 in June to 0.46 in July ($P < 0.01$). Digestibility (%) of the UIP in smooth bromegrass decreased from 38.6% in June to 27.1% in July, due to the increase in IDP. Even though the increase in IDP was larger in BFT in July, more UIP was flowing from the rumen, resulting in a tendency for the digestibility (%) of UIP to increase from 21.1 in June to 25.1 in July ($P = 0.07$).

Experiment 2

Adding heat during the drying process increased the flow of UIP from the rumen as the UIP of alfalfa increased with exposure to heat (Table 2). The UIP (% DM) of freeze-dried, sun-cured, and dehydrated alfalfa was 1.84, 2.10, and 3.13, respectively ($P < 0.01$). Indigestible protein (% DM) was not different in freeze-dried (1.57) and sun-cured alfalfa (1.56); however, the IDP of dehydrated alfalfa (1.66) was increased slightly above the other two drying methods ($P < 0.01$). There was a tendency ($P = 0.06$) for greater total tract IDP in alfalfa fertilized with high N (1.62) than with the low N level (1.57); however, this small increase in IDP did not adversely affect the digestibility of the UIP (data not shown).

The net effect of heat treatment on the digestibility of UIP was quite large as a result of the differences in UIP flowing to the small intestine. The digestibility (%) of the UIP in freeze-dried, sun-cured, and dehydrated alfalfa were 14.7, 25.6, and 46.4 ($P < 0.01$). The small increase in the IDP for dehydrated alfalfa was offset by the larger increase in the UIP flowing from the rumen and resulted in the higher digestibility of UIP. Compared to freeze-dried alfalfa, dehydrated alfalfa supplied 444% more protein in the small intestine.

Accurate UIP values of forages determined using appropriate incubation times of feeds in the rumen is a critical component in the determination of the digestibility of the UIP in forages. The heat-treated alfalfa used in the present study shows the effect of decreased ruminal degradability on lower intestinal tract digestibility values of the UIP fraction. The constant digestibilities used in current protein evaluation systems may be appropriate for concentrate feeds; however, these values appear to be too high for forages. The digestibility of the UIP in the forages in the current study was low, varying from 14.7% to 46.4%.

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